

BONUS BIO-C3

Biodiversity changes: causes, consequences and management implications

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BIO-C3 overview

The importance of biodiversity for ecosystems on land has long been acknowledged. In contrast, its role for marine ecosystems has gained less research attention. The overarching aim of BIO-C3 is to address biodiversity changes, their causes, consequences and possible management implications for the Baltic Sea. Scientists from 7 European countries and 13 partner institutes are involved. Project coordinator is the GEOMAR Helmholtz Centre for Ocean Research Kiel, Germany, assisted by DTU Aqua, National Institute of Aquatic Resources, Technical University of Denmark.

Why is Biodiversity important?

An estimated 130 animal and plant species go extinct every day. In 1992 the United Nations tried countering this process with the "Biodiversity Convention". It labeled biodiversity as worthy of preservation – at land as well as at sea. Biological variety should not only be preserved for ethical reasons: It also fulfils key ecosystem functions and provides ecosystem services. In the sea this includes healthy fish stocks, clear water without algal blooms but also the absorption of nutrients from agriculture.

Biodiversity and BIO-C3

To assess the role of biodiversity in marine ecosystems, BIO-C3 uses a natural laboratory: the Baltic Sea. The Baltic is perfectly suited since its species composition is very young, with current salt level persisting for only a few thousand years. It is also relatively species poor, and extinctions of residents or invasions of new species is therefore expected to have a more dramatic effect compared to species rich and presumably more stable ecosystems.

Moreover, human impacts on the Baltic ecosystem are larger than in most other sea regions, as this marginal sea is surrounded by densely populated areas. A further BIO-C3 focus is to predict and assess future anthropogenic impacts such as fishing and eutrophication, as well as changes related to global (climate) change using a suite of models.

If talking about biological variety, it is important to consider genetic diversity as well, a largely neglected issue. A central question is whether important organisms such as zooplankton and fish can cope or even adapt on contemporary time scales to changed environmental conditions anticipated under different global change scenarios.

BIO-C3 aims to increase understanding of both temporal changes in biodiversity - on all levels from genetic diversity to ecosystem composition - and of the environmental and anthropogenic pressures driving this change. For this purpose, we are able to exploit numerous long term data sets available from the project partners, including on fish stocks, plankton and benthos organisms as well as abiotic environmental conditions. Data series are extended and expanded through a network of Baltic cruises with the research vessels linked to the consortium, and complemented by extensive experimental, laboratory, and modeling work.

From science to management

The ultimate BIO-C3 goal is to use understanding of what happened in the past to predict what will happen in the future, under different climate projections and management scenarios: essential information for resource managers and politicians to decide on the course of actions to maintain and improve the biodiversity status of the Baltic Sea for future generations.

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I. Executive Summary

The deliverable shed light on the direct and indirect food-web effects of increasing abundance and expanding ranges of certain invasive non-indigenous species on native populations and ecosystem functioning. The results support predictions about impact of invasive and non-indigenous species performed in WP3 and WP4. Focus was given on the invasive species which are expanding along the coastal ecosystems, affecting a range of trophic interactions including direct predation, competition with native species and population regulation by their predators and parasites. Round goby (*Neogobius melanostomus*) was the most widely studied invasive species due to its wide range and potential large effects on the food web functioning. In addition, the role of Harris mud crab (*Rhithropanopeus harrisi*), grass prawn (*Palaemon elegans*), red-gilled mud worm (*Marenzelleria* spp.), and comb jelly (*Mnemiopsis leidyi*) were assessed by field sampling, laboratory experiments and stable isotope analysis. *Mnemiopsis* results have been partly utilized also in 1.3 and 2.2 reports concerning adaptation potential and impacts on herring larvae.

The diet and prey preference of the round goby (*Neogobius melanostomus*) and the potential competition with native species was studied in various locations throughout the Baltic Sea. Round goby seems to be an opportunistic predator feeding on a variety of prey species, the diet depending on the local prey community available. Furthermore, the deliverable gained knowledge on the predators and parasites of the round goby, which could have potential to regulate the populations and further spread in the invasive range in the Baltic Sea. With these results the role of the round goby in the Baltic Sea food webs can be assessed more accurately than before.

Another invasive species the deliverable shed light on was the Harris mud crab (*Rhithropanopeus harrisi*) which have been part of the fauna in the southern parts of the Baltic Sea for more than a century but which suddenly begun spreading to new areas in late 2000's. The impacts of this species were investigated in newly invaded areas in the Northern Baltic Sea, in Finland and in Estonia. The results show that Harris mud crab has established also in the northern areas and shows to prefer the keystone habitats. Their isotopic signature places them amongst second-degree consumers and the experiments show that they predate preferentially on grazers, especially gastropods, which may have implications on the health of the coastal ecosystems. Furthermore, in areas where the crabs are abundant, they are also experiencing predation from local fish species. The Harris mud crab has therefore found and established in a niche and has become a part of the coastal systems.

The third invasive species studied was the Grass prawn *Palaemon elegans*, which has been colonizing the Baltic Sea since early 2000's. In the southern Baltic their success may have contributed to a decline of the native shrimp *P. adspersus*, but similar pattern has not been observed in the northern Baltic Sea range yet. In addition, based on field samplings in the north both species are abundant in similar environments and in the experiments it was observed that the non-native *P. elegans* showed more aggression towards *P. adspersus* than vice versa. If competition will be more intense in the future, there might be impacts on the native shrimps also in the north. The role of *P. elegans* as a prey was also studied. It was found to be a frequent prey for perch and cod in the central Baltic Sea whereas in the north perch didn't eat this novel prey. It seems that fish may need more time to find this prey.

The deliverable also gained new knowledge on the benthic invasive *Marenzelleria* spp. polychaetes which are among the few invaders potentially impacting the Baltic Sea positively. They have an ability to burrow much deeper into the sediments than native species and can therefore oxygenate sediments and impacting also biogeochemical processes such as nutrient cycling. In the Central Baltic, in the Vistula Lagoon they have

begun to dominate the benthos and significantly altered the benthic habitats. Species distribution modelling indicates that the abundance of *Marenzelleria* ssp. in shallow water sediments makes them potential prey for coastal fish and in these areas competition with native species is likely. Furthermore, they have potential to increase phosphorus retention in bottom deposits due to deeper oxygen penetration into sediments and formation of a deeper oxidized layer. This may contribute to significant changes in nutrient cycling.

The deliverable also reports on the effective strategy of the non-indigenous comb jelly *Mnemiopsis leidyi*, which has been present in the Northern Europe since 2005. We show for the first time that low winter temperatures have a significant effect on the range expansion of *M. leidyi* and re-seeding of animals from high abundance hot-spots has been documented to be extremely fast with more than 2,000 km year⁻¹. Also, we show that *M. leidyi* in invasive sub-populations show selection for traits to optimize its population growth, compared to native sub-populations which optimize for individual life time reproductive success. Combined, these two major findings show that *M. leidyi* is a very potent invasive species due to its fast population growth but also its swift re-colonization following local extinctions.

II. Introduction

Biodiversity changes are intimately linked to food web functioning. Changes in species and population distribution and abundance partly reflect the natural dynamics of habitats in the Baltic Sea and partly the growing importance of multiple human drivers (the focus of the task 3.1) including introductions of non-indigenous species. Biodiversity influences food web structure, ecosystem functioning, and stability. Low local species richness and resulting tight trophic links make the Baltic Sea food webs susceptible to impacts of invasion of non-indigenous species. It has been shown and predicted that disturbed aquatic systems are especially susceptible to invasions and appear to be especially vulnerable. Compositional changes due to increasing arrival and establishment of non-indigenous species have been documented for the Baltic Sea and colonization will depend on their physiological adaptation to low salinities, which currently limits invasion. Therefore, food web processes need to be investigated from a physiological and competition aspect to understand the cumulative effects of non-indigenous species and climate change for structure, functioning and consequently biodiversity of the ecosystem.

This task focused on direct and indirect food-web effects of increasing abundance and expanding ranges of invasive non-indigenous species as well as habitat engineering through these effects on native populations. The results support predictions about impact of invasive non-indigenous species performed in WP3 and WP4. Focus was given on the invasive species which are expanding along the coastal ecosystems, affecting a range of trophic interactions including direct predation, competition with native species and potential population regulation by their predators and parasites. These invasive species provide new trophic links with organisms ranging from mussels and crustacean grazers to fish (e.g. cod, perch) and birds (cormorants), and may indirectly interfere with native species. Round goby (*Neogobius melanostomus*) was the most widely studied (P3, P5, P6, P7, P8, P11, P13) invasive species due to its wide range and potential large effects on the food web functioning. In addition, the role of Harris mud crab (*Rhithropanopeus harrisi*) (P6, P7, P13), grass prawn (*Palaemon elegans*) (P6, P7, P8), red-gilled mud worm (*Marenzelleria* spp.) (P5, P8, P13), and comb jelly (*Mnemiopsis leidyi*) (P1, P2, P3, P5, P7) were assessed by field sampling, laboratory experiments and stable isotope analysis. The research covered the whole Baltic Sea area with special focus areas being the Archipelago Sea, Gulf of Finland, Gulf of Riga, South-Eastern Baltic Sea, Bornholm Basin and Western Baltic.

III. Core Activity

III.1. Impacts of the round goby, *Neogobius melanostomus*

i) Abstract /highlights

The main focus of the work was the potential direct and indirect impacts of the round goby (*Neogobius melanostomus*) on the Baltic Sea food webs. Most efforts were directed into investigating their feeding in the Baltic Sea, of which very little information was available before. The diet and prey preference of the round goby the potential competition with native species was studied in various locations throughout the Baltic Sea. Furthermore, the project gained knowledge on the predators and parasites of the round goby, which could have potential to regulate the populations and further spread in the invasive range in the Baltic Sea. With the results, the role of the round goby in the Baltic Sea food webs can be further assessed.

i) Progress and (if applicable) deviations from the original work-plan

Most of the work is either published or very close to submission. Some manuscripts are currently being prepared.

ii) Deviations from the work-plan

None

iii) Introduction

The round goby originates from the Ponto-Caspian area and has spread to the Baltic Sea through the channel system in Europe (Panov et al. 2007) likely using ship traffic as a vector (Moskalkova 1996). The round goby was first observed in the Baltic Sea in the Gulf of Gdansk, Poland in 1991 (Skora and Stolarski 1993) and in the last few years it has invaded even the northernmost parts of the Baltic Sea (Kotta et al. 2016).

Previous studies have shown that the round goby is an aggressive, territorial and voracious benthivorous fish (Charlebois et al. 1997) observed to have profound impacts on the ecosystems especially where numerous (e.g. Laurentian Great Lakes and Gulf of Gdansk, Poland)(Dubs and Corkum 1996, Lauer et al. 2004, Balshine et al. 2005, Lederer et al. 2006, Almqvist et al. 2010). The round goby is a generalist predator reported to feed primarily on bivalves when available (Skora and Rzeznik 2001, Karlson et al. 2007, Rakauskas et al. 2008, Raby et al. 2010, Järv et al. 2011), but from majority of the Baltic Sea, information on their diets was lacking prior to this project.

Round goby have potential to compete with economically important fish species such as flounder in regions where they are abundant as indicated by resource overlap (Karlson et al. 2007, Ustups et al. 2016). Due to their generalistic feeding, there is potential for intense resource competition, especially where preferred prey is limited.

In regions where round goby have become abundant, they have themselves become important prey items to both avian and fish predators (Jakubas 2004, Dietrich et al. 2006, Almqvist et al. 2010). This project aimed to investigate the potential predators of the round goby in various locations in the Baltic Sea in more detail. Furthermore, very little was known about the parasites of the round goby. This project produced a study comparing the parasite load of the round goby throughout the Baltic Sea.

The aim of the work reported here was to provide information on the role the round goby plays in the coastal food webs in the Baltic Sea and fill in gaps in existing knowledge. With the results of the studies, their impacts in each location can be assessed in more detail.

iv) Methods and results

Feeding of the round goby and competition with native species

Laboratory experiments were conducted by UT-EMI (P6) to study the feeding and prey preference of the round goby in the north-eastern Baltic Sea (Nurkse et al. 2016). The results showed that the round goby is able to effectively consume a diverse variety of prey when given the choice between dominant benthic invertebrates: bivalves (*Macoma balthica*, *Mytilus trossulus*, *Cerastoderma glaucum*) and amphipods (*Gammarus* spp.). In contrast consumption of the gastropod (*Theodoxus fluviatilis*) was very low in all provided combinations. Nevertheless, the round goby had no statistically significant preference towards any of the prey taxa. The round goby exhibited sizespecific consumption of *M. trossulus*, with smaller individuals being consumed at least 25% more than larger size classes. In addition elevated prey density resulted in higher consumption of prey by the fish. The broad diet suggests that shifting densities of benthic invertebrate prey has little influence on the further dispersal of the round goby in the Baltic Sea as the species is potentially able to switch between several native invertebrate taxa. This opportunistic feeding behaviour has likely favoured the invasion and ensured success of the species in the invaded ecosystem.

The diets and prey preference of the round goby were studied by field sampling and laboratory analysis by SYKE (P7) in three locations in the Baltic Sea, representing very different biotic (including prey field) and abiotic conditions (Puntila et al. in prep). The results show that round goby is very flexible and non-selective feeder, preying usually on the most abundant prey. The availability of the prey in each of the locations is reflected to the stomach contents. In areas where bivalves are abundant they often are the most prevalent prey, but in other locations round goby feed variably on other benthic prey such as gastropods and even on barnacles (Fig 1). Contrary to the original hypothesis about round goby uniformly preferring bivalves, the selectivity analysis showed that the round goby rarely show preference towards any prey or they show preference also towards other than bivalve prey. In Guldborgsund, Denmark the small (<10cm) and medium (10-15cm) round goby showed positive selection towards *Mytilus trossulus* and large (>15cm) towards *Parvicardium* spp. Regardless of the high abundance of polychaetes in the benthic samples, fish in all size classes showed avoidance towards them. In the Gulf of Gdansk, prey preference also varied between size classes. Small round goby (<10cm) preferred gastropods and amphipods. The medium sized (10-15cm) round goby preferred mainly *Cerastoderma glaucum* and the largest ones (>15cm) *Mytilus trossulus*. In Mariehamn in the northern Baltic, selection was strongest towards *Amphibalanus improvisus* in all size classes. Other prey types were either avoided or consumed relative to their abundance in the benthic samples. Finally, the prey appears to have very little impact on the condition of the fish indicating that round goby populations are not relying on presence of any specific prey organisms and are capable of maintaining healthy populations under different environmental conditions.

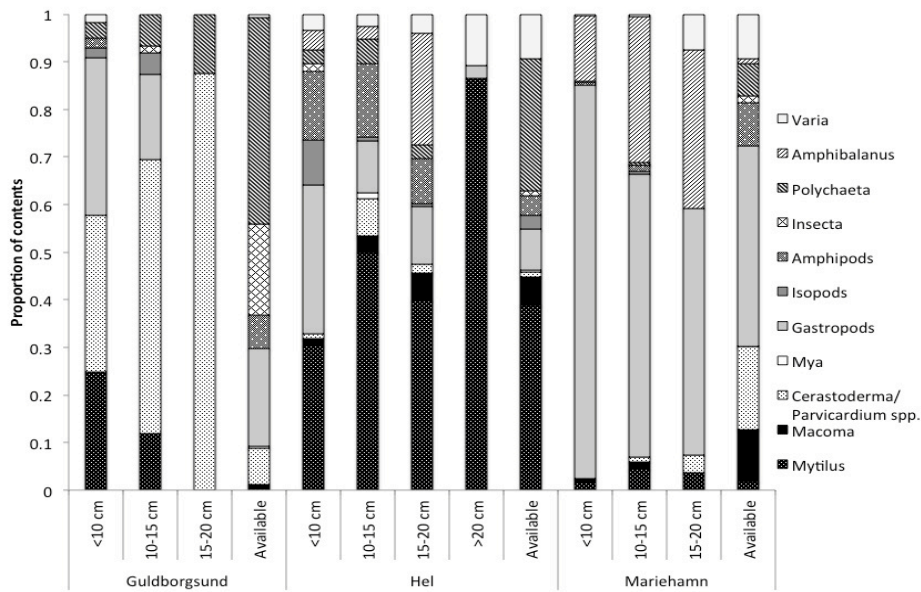


Figure 1. Stomach contents of the round goby in three sampling areas around the Baltic Sea.

The trophic interactions between round goby and the resident fauna were studied in the western Baltic Sea by TI-OF (P11). The results indicated a size-dependent feeding behaviour of the gobies comparable with the feeding ecology observed within their native range. While the amount of ingested crustaceans decreased for growing juveniles, they become more and more molluscivorous. Furthermore, comparative catches between vegetated and unvegetated sites within the study area Greifswald Bay demonstrated a clear preference towards complex and structured habitats. However, the vegetated areas were primarily used by juvenile individuals while adults are rare at these sites. We hypothesised that distinct life stages of round goby utilize different habitats in Greifswald Bay; while juveniles use the vegetated areas as nursery grounds, adults might aggregate in other areas which offer more appropriate spawning substrate such as stone fields. A manuscript about the study is in preparation (Henseler et al., in preparation).

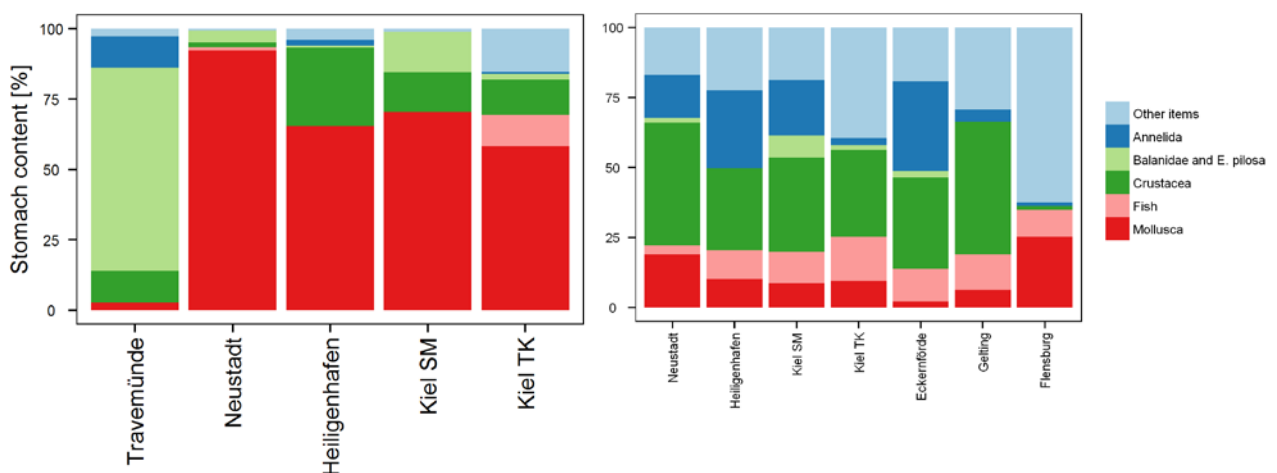


Figure 2. Stomachs contents of round goby (left panel) and black goby (right panel) in the western Baltic Sea.

Diet of round goby was also studied by field sampling in the western Baltic Sea by UHH-IHF (P3) (Mattern 2015). Stomachs were collected from the two dominant goby species, the resident

black goby and the invasive round goby using hand line surveys. Diet analyses revealed that round goby at the northern German Baltic coast specialized either on barnacles or molluscs, while black gobies had a broader prey spectrum including crustaceans, annelids and molluscs (Figure 2.). Furthermore some black goby individuals were specialized on fish. A low dietary overlap was calculated for the two goby species, which suggests that other factors such as competition for habitat and nesting sites have caused the disappearance of black gobies in Travemünde.

The effects of round goby on herring recruitment was studied in Greifswald Bay, a major spawning area for Atlantic herring (*Clupea harengus*) in the western Baltic Sea by TI-OF (P11). We analysed if and to what extent round goby might prey on herring eggs during the short but intensive herring spawning season in spring. Earlier investigations have shown a significant predation of native three-spined stickleback (*Gasterosteus aculeatus*) on the eggs of herring. Considering the recent spread of round goby in the study area and the ease to prey on demersal herring eggs we expected an intensive predation on the eggs. However, we observed a clear spatio-temporal mismatch between round goby and herring egg occurrence in one of our main study sites in Greifswald Bay. Our station "Gahlkow" is characterized by an extended sandy littoral which is densely covered with submerged aquatic vegetation (SAV), consisting mainly of pond weeds (Potamogetonaceae) and seagrass (Zosteraceae). While small and medium sized round goby dominate the small fish community at Gahlkow in summer and autumn (August-October) the community is dominated by *G. aculeatus* during the spring spawning season (March-May) with almost no round goby present on the spawning bed. We therefore extended our focus to include areas within the bay which are used as spawning grounds according to earlier observations but also offer a more suitable habitat for round goby. This includes rocky areas which cannot be sampled with the usual active sampling gears (e.g. beach seines). Common passive sampling gears, however, are limited in their suitability for investigations on trophic interactions since uncertainties and biases arise from the unknown retention time of predators in the gear/trap. Furthermore, in the recent years our study area was subject to an extraordinary intense mass development of filamentous brown and green algae. These algae cause a notable clogging of fykes and other passive nets significantly reducing their catchability and inappropriately increasing the effort required for the maintenance of the nets.

In 2016, we therefore continued our trials with modified passive sampling devices to cope with this problem and developed a promising device. Considering behavioural traits of round goby, we tried to build special traps offering hiding places which the fishes can freely enter and leave. This approach avoids biases caused by baited traps or unknown retention times of the fishes within the traps. First prototypes consisting of car tyres combined with a net at the bottom (Figure 3A) were successfully catching round goby but frequent damages of the net caused by the rocky sea floor required a considerable time for repairs after each use. We therefore tried to use a more robust prototype consisting of a modified eel trap with one open entry, enabling the gobies to enter and leave the tube freely (Figure 3B). To catch gobies, the tube is lifted quickly with the open side ahead. From early summer of 2016 on, we were able to reliably catch round goby using this method. However, at this point, the spawning season of herring was almost over but this approach will be used in future investigations on round goby feeding ecology in Greifswald Bay.

We conclude, that predation by round goby probably plays a rather minor role in herring egg mortality in the western Baltic Sea since many of the important spawning beds in Greifswald Bay are characterized by a less suitable habitat type (sand + SAV but no rocks). However, it cannot be excluded, that a potential overlap of round goby and herring eggs in more structured habitats

might result in a predation which should be investigated in the future. Therefore we supervise a master thesis about the nutrient ecology of round goby with the focus on potential feeding on herring eggs in spring 2017. To do so, we conduct different lab experiments to test whether herring eggs are preferred compared to other nutrient items. We expect the first results this summer after the herring spawning season.

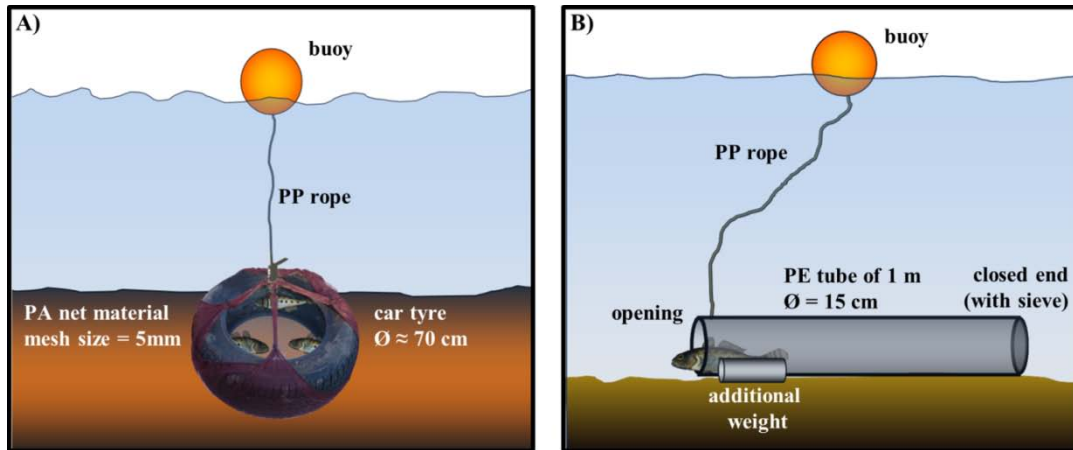


Figure 3: Passive sampling devices developed to catch round goby in Greifswald Bay for stomach sampling. Both prototypes enable the gobies to enter/leave freely avoiding stomach sampling biases caused by unknown retention times within the traps A) Device based on a car tyre. B) Modified eel pot with additional weights. PP = polypropylene; PE = polyethylene; PA = polyamide (nylon).

The results of different supervised master thesis's were summarized to show the potential top down and bottom up effect of round goby in the Pomeranian Bay (Oesterwind et al. 2017). Regarding the top down studies the stomach content analysis of round goby show that *N. melanostomus* consumed a variety of prey organisms including polychaetes, insects, crustaceans, bivalves and gastropods.

The results from the bottom up analysis show, that *Scophthalmus maximus* and *Gymnocephalus cernuus* do not feed upon *N. melanostomus* within the study area. While *Perca fluviatilis* and *Sander lucioperca* preyed upon *N. melanostomus* beside other prey species. The Index of Relative Importance (IRI) shows that *N. melanostomus* became an important prey for *S. lucioperca* and *P. fluviatilis* during the last years within the study site. In addition cormorants' pellets were analysed at two different locations. The study illustrates that the occurrence of *N. melanostomus* within the pellets increased significantly over the last years.

Effects of round goby occurrence on the biomass densities of the flounder (*Platichthys flesus*), which constitutes one of the commercially important and most common flatfish in the region were investigated by NMFRI (P5). Data from the Baltic International Trawl Surveys (BITS) conducted in the 1st and 4th quarter of the years 2003-2017 were used in the study. Based on the number of fish per length class and obtained weight-length relationships biomass densities of flounder were estimated. Information on first occurrence of round goby around the Baltic Sea was interpolated for the study area to simulate effect of this species invasion. In order to incorporate natural sources of variation in the flounder biomass, hydrological conditions measured in situ (depth, bottom temperature and salinity) together with modelled data (slope of seabed, mean annual current velocity, type of sediments) were incorporated in the generalized additive models (GAMs) developed for each quarter. Year effect and effect of round goby occurrence were added as additional factors. Models for 1st and 4th quarter explained 20.7 % and 50.7% of deviance, respectively. All variables included in the models were statistically

significant ($p < 0.05$) with exception of sediments and slope in model for 1st and 4th quarter, respectively. According to obtained predictions flounder biomass was driven mainly by depth. Modeling results revealed negative effects of round goby occurrence on flounder biomass. Significant differences ($p < 0.001$) were found between hauls conducted before and after simulated first occurrence of round goby. These results may have importance for ecosystem-based management of the commercially exploited resources of the Baltic Sea and risk assessments for invasive species (Smoliński S. in prep.).

The trophic position and niche space of the round goby was studied comparatively between old invasive area, Gulf of Gdansk, Central Baltic Sea and new invasive area, Mariehamn, Åland, Northern Baltic Sea by ÅA (P13, P7). Sampling was conducted during the reproductive season in 2014 at both sites. The whole benthic community in Mariehamn was sampled for stable isotope analysis. In Hel, the sampling was concentrated on round goby and the benthic prey they may consume. A cluster analysis based on Euclidean distances was performed on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of round goby in Mariehamn and Hel to determine relevant size class groupings of round goby, which were used in the subsequent analyses and graphic representations. Furthermore, clustering was used to assign the round goby size classes into trophic levels. The trophic positions (T_p) were calculated to the round goby from both sampling sites. In addition, Stable Isotope Bayesian Ellipses analysis were applied to the most abundant (in our data set) benthic fish to investigate trophic niche overlap between the round goby and other fish species in Mariehamn, as well as between eelpout and round goby in Hel. The results show that based on the clustering analysis the round goby is placed as second order consumer. The ^{15}N (‰) ratio implies that round goby in Mariehamn have higher trophic level than in Hel, potentially due to different prey items in these areas. Based on the Stable Isotope Bayesian Ellipses analyses, the round goby experiences significant intraspecific competition as well as competition with other predatory fish (Fig. 4) (Herlevi et al. in prep).

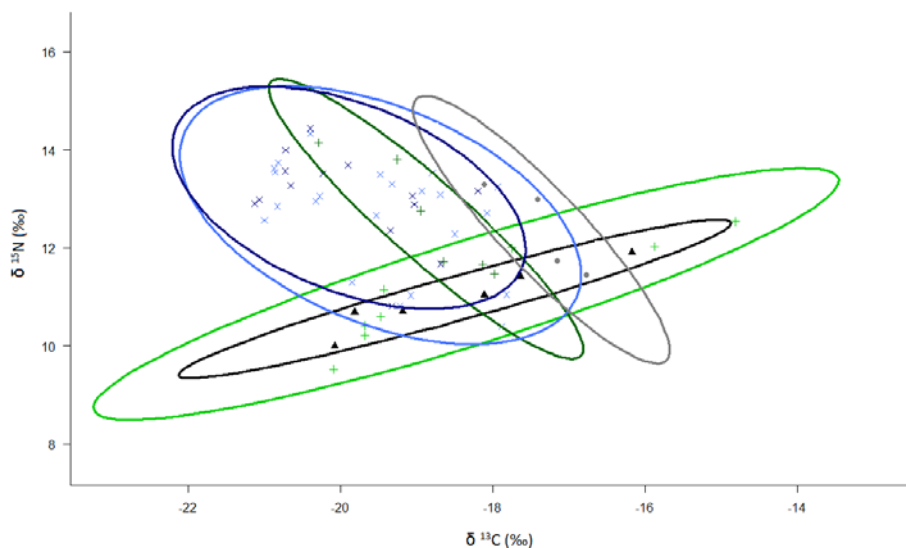


Figure 4. The bivariate isotope data ($\delta^{13}\text{C}$ (‰) and $\delta^{15}\text{N}$ (‰)) and their respective standard ellipses (SEAc) for four benthic fish species in Mariehamn. The prediction ellipses have been scaled to contain 95% of the estimated data. ●: *Blicca bjoerkna*. ×: *Neogobius melanostomus*. +: *Perca fluviatilis*. ▲: *Rutilus rutilus*. Large and small perch and round goby are shown with different colors: light green= small perch. dark green= large perch. light blue= small round goby. navy=large round goby.

Impact of round goby on local populations and food web was studied in the Lithuanian coast, SE Baltic Sea by KU-CORPI (P6). Work in 2015 focused on the determination of the diet composition

and feeding activity of round goby in relation to body size, sex and reproductive cycle (Skabeikis and Lesutienė, 2015). Specific dietary analyses showed that benthic fauna, particularly newly settled generation of epibenthic mollusks in autumn, is likely under a strong predatory pressure of the round goby, and recruitment success of the blue mussel may be considerably affected by the intense round goby consumption of newly settled juveniles. This can explain dramatic reduction of *M. trossulus* abundance and biomass in the Lithuanian coastal waters in the recent past. Round goby predation also has a great potential to directly and indirectly affect diversity and abundance of many other benthic invertebrate species living in association with colonies of the blue mussel.

Diet composition, feeding activity and feeding niche overlap between round goby and wintering benthophagous long-tailed duck (*Clangula hyemalis*) were studied during different round goby invasion phases by KU-CORPI (P8) (Skabeikis et al. in press). In total guts of 510 round goby and gizzards of 107 long-tailed ducks, collected in the 20 km stretch of the Lithuanian coastal rocky biotope during 2002-2016, were analysed. Feeding patterns of round goby and long-tailed duck changed dramatically along with declining blue mussel biomass, which decreased consistently from round goby establishment ($2.3 \pm 1.5 \text{ kg m}^{-2}$) to expansion ($0.001 \pm 0.001 \text{ kg m}^{-2}$) and remained low ($0.03 \pm 0.004 \text{ kg m}^{-2}$) during adjustment phase. During establishment, both round goby and long-tailed duck preyed mainly on *Mytilus edulis trossulus*. During expansion, the diet of round goby was dominated by *M. edulis trossulus*, *Crangon crangon* and *Macoma balthica*, while in the last invasion period it shifted to polychaetes. Long-tailed duck changed its diet from epibenthic blue mussels to fish prey during round goby expansion and adjustment phases (Fig. 5).

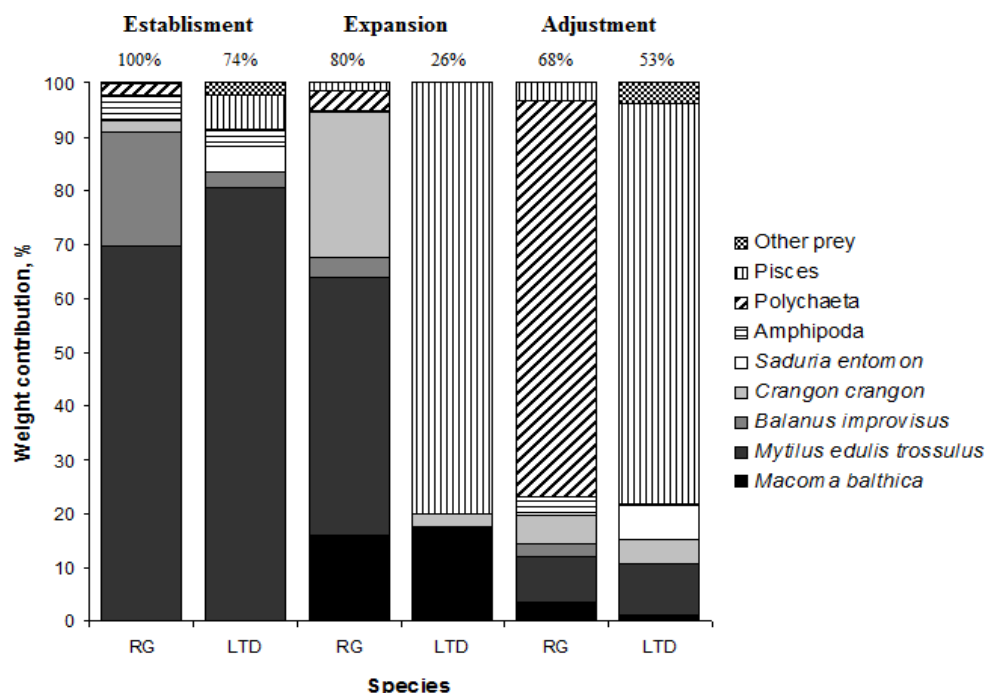


Figure 5. Mass percentage diet composition of round goby (RG) and long-tailed duck (LTD) during different round goby invasion phases. Numbers above the columns indicate feeding efficiency (percentage of full guts/gizzards).

Feeding niche overlap between round goby and long-tailed duck was biologically significant during round goby establishment (0.76), but lost significance during expansion (0.20) and adjustment (0.17) phases after drastic blue mussel decline in natural environment.

In conclusion, round goby predation on epibenthic community directly or indirectly affected populations of many species belonging to different trophic levels and induced major shifts in food web structure and energy flows of the rocky coastal area of SE Baltic Sea (Fig 6).

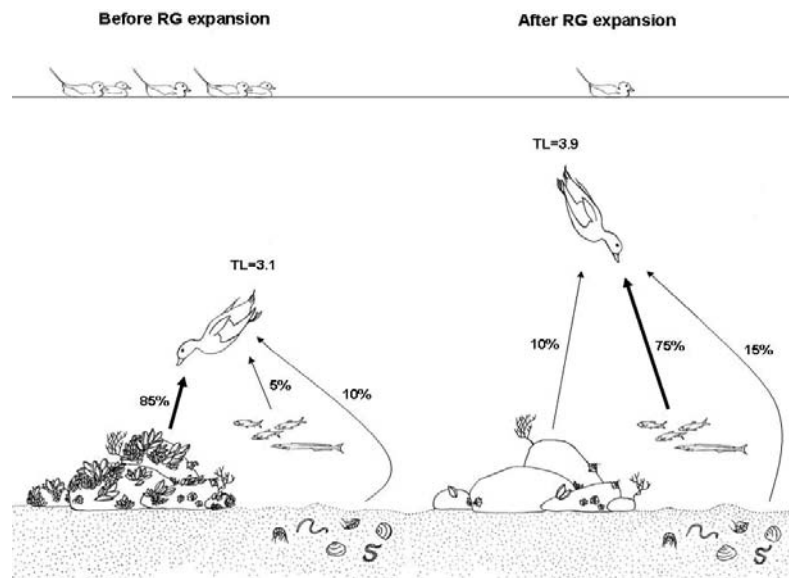


Figure 6. Scheme, demonstrating shifts of the food web and long-tailed duck trophic levels before and after round goby expansion in the rocky coastal habitat of SE Baltic Sea ©S. Karalius.

Potential regulation of the round goby populations by predators and parasites

The role of round goby as prey for cod was studied by stomach sampling in 2015 and 2016 by UHH-IHF (P3). The overall aim was to get an idea when, where, in which depth and at what size are cod preying on round goby. Since the 1990s several studies have examined the diet of eastern Baltic cod stock. However, the only available studies of the western Baltic cod stock were based on stomach samplings in the 1960s till 1980s. Furthermore almost all previous diet studies of Baltic cod were based on stomach samplings in waters deeper than 20 m. Therefore, the role of shallow habitats as feeding grounds for cod is somewhat uncertain. However, the invasive round goby (*Neogobius melanostomus*) lives preferably in these shallow habitats.

A total of 1160 stomachs of cod > 35 cm were collected in both, shallow and deep waters of the western Baltic Sea (SD 22). Cod were caught between February and November 2016 by angling, commercial gillnetting, commercial trawling, during several research surveys conducted by the University of Hamburg (P3), and the Thünen Institute of Baltic Sea fisheries (P11). All stomach samples were analyzed on a species level for fish and most of the rest on taxa level. Additionally length and weight of the prey items were measured (Funk 2017).

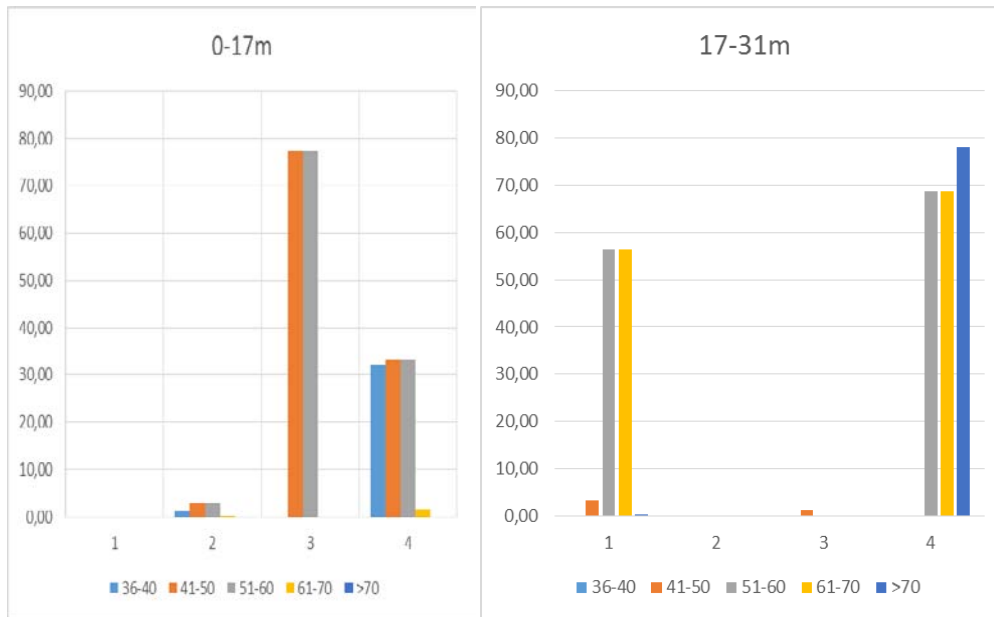


Figure 7. Percentage of round goby in the diet of cod per length class, quarter and depth stratum.

These results highlight, that round goby have become a common prey species for cod (Fig. 7). Cod mainly preys in late autumn and winter on round goby. In summer only a limited number of round gobies were observed in the diets of cod. This might be explained by a lack of spatial overlap between predator and prey in summer. While cod tend to move in deeper colder areas during peak summer time (Funk, 2017), round goby apparently prefer warm, shallow areas. The energetic optimum of round goby was found to be around 26°C (Lee & Johnson, 2005) Higher diet shares of round gobies in the cod stomachs in depths > 17 m were mainly observed in November and February, which might be an indication for temperature dependent movement patterns of round gobies to deeper areas with decreasing water temperature. Similar pattern can be observed in Lake Ontario (Great Lakes USA) where round goby was found down to 130m (April sampling, Walsh et al 2007).

The prevalence of the round goby in local fish diets was also studied in Lithuania by KU-CORPI (P8). Perch was collected in July, while cod in October-November, 2014. Remains of the round goby were found in both small and large perch stomachs (16 and 35 %, respectively) and their proportion in the cod diet was in average ~40% (Fig. 8). Therefore it is apparent that round goby have become important food components of local predators and we can expect some top-down regulating effects of the invader populations in the future (Skabeikis in prep).

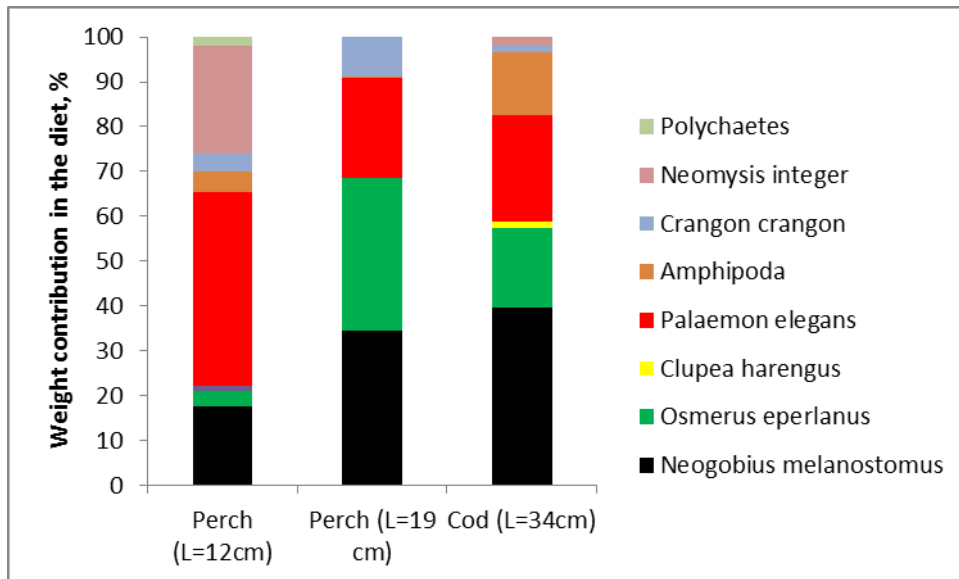


Figure 8. Diet composition of predatory fish (perch and cod) in the coastal stony bottom area (Lithuania).

Infection rates and prevalence of metazoan parasites of the round goby were studied in the Baltic Sea (Herlevi et al. 2016) by SYKE (P7). We aimed at comparing parasite assemblages and infection rates (prevalence and intensity) in different populations across the invasive range in the Baltic Sea (Denmark, Lithuania, Estonia and Finland). Infection rates were 56–60% across all locations except Lithuania (28%). However, the parasite assemblages in the sampled populations were dissimilar, each location having unique parasites. In addition, many of the parasites were generalists commonly infecting native fish species. Based on the results of this study and those previously conducted in the Baltic Sea, the round goby has not retained parasites from its area of origin, but instead has been successively colonized by native generalist parasites. There is also no evidence of round goby introducing new parasites to the Baltic Sea. Although variable, overall parasite richness is still quite low around the Baltic compared to the native areas (34 vs 71 taxa, respectively). Also, prevalence and mean infection intensities in the Baltic Sea are significantly lower than in the native areas. Parasite load did not appear to impact the round goby negatively. Therefore, the invasion success of the round goby in the Baltic Sea can at least partly be attributed to enemy release, in this case shedding a significant proportion of their native parasite load.

i) Recommendations

Based on the results of the project it appears that the round goby has managed to establish successfully in very different environments and is efficiently utilizing the available resources. In the numerous studies conducted within the task, the variability of the species diet is evident. Due to this variability the impacts are very location-specific.

The round goby competes with native species over various limited resources, but in this task competition over prey, was studied in more detail. Competition is intense especially during the first invasion stages causing changes in the diets of both native species and the round goby.

Furthermore, the round goby itself has become a prey for native predators. Avian predators such as cormorants as well as large piscivorous fish such as cod, sander and perch have begun to utilize the species. Also, the parasites in the new range have found this new species. They have

been infected by generalist parasites in the Baltic Sea although the infection intensity remains lower than in most native species or the round goby in other areas.

The research conducted within BIO-C3 can further be used in assessing and quantifying the impacts of the round goby in the whole Baltic Sea scale. Furthermore, they provide information that can be utilized by follow up projects and other WPs of the project (e.g., food web modelling studies).

ii) List of publications:

Funk, S. 2017. Depth-specific patterns in distribution and food intake of cod (*Gadus morhua*) in the western Baltic Sea; Master thesis, Institute for Hydrobiology and Fisheries Science University of Hamburg. (P3)

Henseler, C., Kotterba, P., Moll, D., Winkler, H., Oesterwind, D. (in prep): Integration of invasive round goby into the resident community of a shallow littoral ecosystem. (P11)

Herlevi, H., Puntila, R., Kuosa, H. & Fagerholm, H-P. 2016: Infection rates and prevalence of metazoan parasites of the non-native round goby (*Neogobius melanostomus*) in the Baltic Sea. *Hydrobiologia* DOI 10.1007/s10750-016-3062-6. (P13 & P7).

Herlevi, H., Aarnio, K., Puntila, R., Bonsdorff, E. (in prep): The food web positioning and ontogenetic diet shifts of the non-indigenous round goby in a recently established population in a low-diversity system in the northern Baltic Sea. (P13 & P7)

Mattern, S. 2015. Spread of invasive round goby (*Neogobius melanostomus*) and food competition with native black goby (*Gobius niger*) in the Western Baltic Sea; Master thesis, Institute for Hydrobiology and Fisheries Science, University of Hamburg. (P3)

Nurkse, K., Kotta, J., Orav-Kotta, H. & Ojaveer, H. 2016. A successful non-native predator, round goby, in the Baltic Sea: generalist feeding strategy, diverse diet and high prey consumption. *Hydrobiologia* (DOI 10.1007/s10750-016-2795-6). (P6)

Oesterwind, D., Bock, C., Förster, A., Gabel, M., Henseler, C., Kotterba, P., Menge, M., Myts, D., Winkler, H., (2017). Predator and prey; the role of the round goby *Neogobius melanostomus* in the western Baltic. *Marine Biology Research*, 13 (2): 188 – 197. (P11)

Puntila, R., Herlevi, H., Arciszewski, B., Lozys, L., Behrens, J. and Ojaveer, H. (in prep): Feeding preferences of the invasive Round goby (*Neogobius melanostomus*) in the Baltic Sea. (P6, P7, P2, P13)

Skabeikis, A. and Lesutienė, J. 2015: Feeding activity and diet composition of round goby (*Neogobius melanostomus*) in the coastal waters of SE Baltic Sea. *Oceanological and Hydrobiological Studies* 44(4): 508-519. (P8)

Skabeikis, A., Lesutienė, J. Morkūnė, R. et al. (in prep): An effect of round goby invasion to mussel beds and its consequences to winter diet of the long-tailed duck. (P8)

Skabeikis A. PhD theses (in prep): The role of round goby (*Neogobius melanostomus*) in the food web of SE Baltic Sea open coast food web. (P8)

Smoliński S. (in prep). Effect of non-indigenous round goby (*Neogobius melanostomus*) on the native European flounder (*Platichthys flesus*) biomass density in the southern Baltic Sea. (P5)

III.2. Impacts of the Harris mud crab, *Rhithropanopeus harrisi*

ii) Abstract /highlights

The invasive Harris mud crab had been part of the fauna in the southern parts of the Baltic Sea for more than a century when the species suddenly begun spreading to new areas in late 2000's. The impacts of this novel species were investigated in Finland and in Estonia. The results show that Harris mud crab has established in productive coastal ecosystems and preferentially occupy important keystone habitats. Their isotopic signature places them amongst second-degree consumers and the experiments show that they predate preferentially on grazers. Furthermore, in areas where the crabs are abundant, they are also experiencing predation from local fish species such as sculpins and perch. The Harris mud crab has therefore found and established in a niche and has become a part of the coastal systems. They are exerting predatory impacts on certain native species, especially gastropods, which may have implications on the health of the coastal ecosystems.

v) Progress and (if applicable) deviations from the original work-plan

Field and laboratory work has been completed and manuscripts are close to submission or already published.

vi) Deviations from the work-plan

None.

vii) Introduction

The Harris mud crab is the most widespread brachyuran crab species in the world, and has invaded at least 20 countries (Roche and Torchin 2007). It established in the southern parts of the Baltic Sea long time ago: they arrived in the Kiel channel already in 1936 (Schubert 1936) and to the Baltic Sea, Germany and Poland in the early 50's (Demel 1953). The Harris mud crab begun another invasion phase in 2000's when it was observed in Lithuania in 2001 (Bacevicius and Gasiunaite 2008), Finland in 2009 (Karhilahti 2010) and Estonia in 2011 (Kotta and Ojaveer 2012).

The Harris mud crab is considered highly omnivorous, feeding on detritus and plant material, as well as, mobile crustaceans depending on the habitat they occupy (Czerniejewski and Rybczyk 2008, Hegele-Drywa and Normant 2009). In the native area, they are mostly scavengers and predation on crustaceans is more common among the small crabs (Odum and Heald 1972). In the Archipelago Sea they have been reported to impact gastropod abundance (Forsström et al. 2015, Jormalainen et al. 2016) and their stable isotope ratio places them among second degree consumers (Aarnio et al. 2015). In the Baltic Sea the crab has invaded many locations with submerged aquatic vegetation and associated rich grazer communities (Nurkse et al. 2015).

In their native area, the Harris mud crab are controlled by parasitic barnacle (rhizocephalan) *Loxothylacus panopei* (Gissler, 1884), which has impacts mainly through disruption of reproduction (Hines et al. 1997). The crabs have, however been able to shed the parasite when entering the invasive range (Fowler et al. 2013), which likely contributes to their success. The Harris mud crabs had rarely been detected in fish stomachs in the Baltic Sea in the older invasive range (Hegele-Drywa and Normant 2009), but recently in the Archipelago Sea fishermen have been reporting finding them (Fowler et al. 2013). The predation pressure is likely impacted by the native predatory fauna present at each location.

viii) Methods and results

Role of the Harris mud crab in the food web

Changes induced by NIS are potentially more pronounced with novel invaders, such as the omnivorous Harris mud crab in the Northern Baltic Sea where it was detected in 2009. It has since established and steadily increased its range. The area lacks any native crab species, providing an excellent opportunity to observe how invasion of a novel species impacts the food web. Aarnio et al. (2015) from ÅA (P13) investigated the position and role of mud crab in the coastal food web by combining spatial and temporal estimates of trophic position using stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), with short-term food- and habitat-choice experiments. They observed a stable pattern in the trophic position between two environmentally different locations in the Archipelago Sea over the production season. The identification of a natural breakpoint in carbon stable isotopes at the size of 12 mm carapace width suggested a presence of an ontogenetic diet shift, which was confirmed by isotope signatures positioning large mud crab among secondary consumers and small individuals with primary consumers. The generalist consumer nature of mud crab and preference for structured habitat was supported by an active habitat and food choice, revealing no preference for prey species (polychaetes versus amphipod), but selection of brown algae over bare mud. To broaden the understanding of the functional role of mud crab, they combined our findings with other known biological and ecological traits of the species and qualitatively compared these to characteristics of other benthic organisms in the northern Baltic Sea, suggesting a general similarity in traits implying that even though the organism is novel to the system, their traits are not.

The food web role of the invasive Harris mud crab was further studied in the Archipelago Sea, Finland by SYKE (P7) (Puntila et al. in prep a and b). Mesocosm experiments and a series of field studies show that the Harris mud crab has established and adapted to the local food web: they appear more predatory than in the native areas and have themselves become a frequent prey item to many native fish species. Apparent prey naïvety (observed in during the experiments) may explain why the Harris mud crab consumes more mobile prey in the Archipelago Sea than in older invasion areas or in the native areas. Results from the experiments also indicate that mud crabs appear to predate preferentially on isopod *Idotea baltica* (Fig. 9) (Puntila et al. in prep a), which is one of the key species in the ecosystem. In the field sampling, abundance changed most among gastropods and mobile crustaceans implying that the Harris mud crab impacts the coastal grazer assemblages (either through direct predation or behavioural responses).

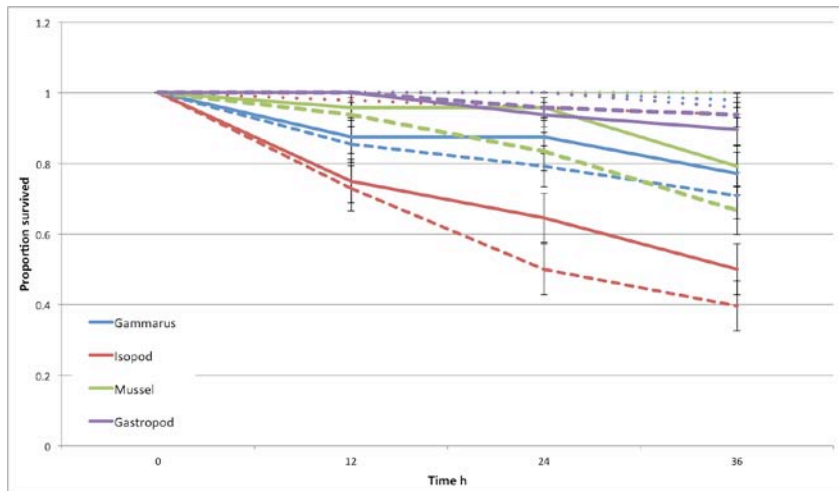


Figure 9. The proportion of prey survived in the mesocosm experiments. The dotted lines indicate no crab controls, solid lines indicate 2-crab treatments and the dashed lines indicate 5-crab treatments.

Based on experimental sampling of fish stomachs in the area as well as local fishermen reports, mud crabs are prey to several local fish species (perch, pikeperch, four-horned sculpin, burbot and roach) (Puntila et al. in prep b). Based on our targeted sampling of local fish communities, four-horned sculpin appears to be the most effective mud crab predator in the area. Of the all four-horned sculpins caught, 85% had consumed mud crabs. In addition, most of them had multiple crabs in their stomachs. Also perch, ruffe and some cyprinids (mostly roach) were recorded consuming mud crabs. The results also show that fish were feeding on a narrower size range of crabs than available in the area, which indicates that mud crabs may acquire shelter in size, as they grow larger (Fig. 10). Despite of the predation, mud crabs have continued to spread further and populations have continued to increase.

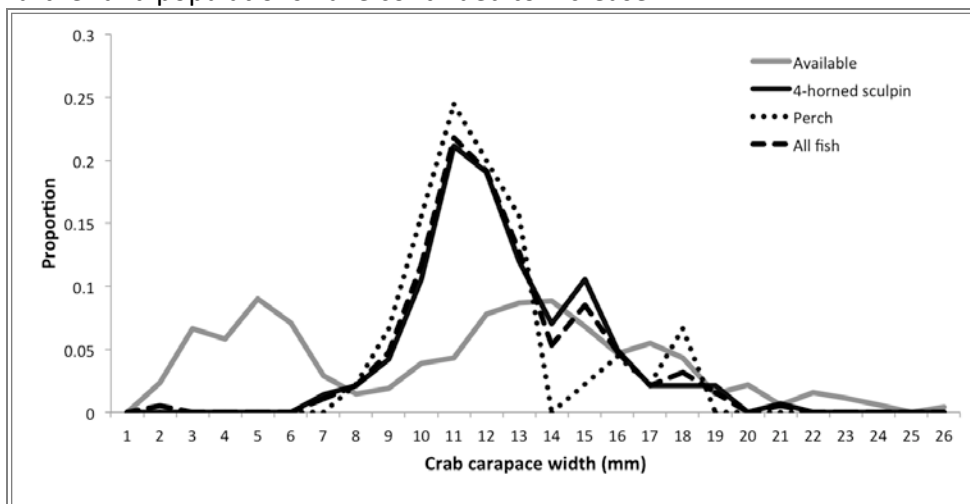


Figure 10. The proportion of different sized crabs in the trap samples, and perch and four-horned sculpin stomachs.

The habitat preference of the Harris mud crab was experimentally evaluated in the Gulf of Riga, the Eastern Baltic Sea by UT-EMI (P6) (Nurkse et al. 2015). The results show that the crabs preferably occupy habitats with vegetated boulders, especially boulders with the perennial *Fucus vesiculosus*. This implies stronger impact of crab in such habitats in the invaded ecosystem. The availability of prey and higher density of the crabs increases the crab's affinity towards otherwise less occupied habitats. Combining 'before-and-after' field observations, censuses of

crab populations and manipulative field experiments, the impacts of the crab on benthic communities was also evaluated. The introduction of the novel predator into an ecosystem previously lacking such ecological function shifted the balance between bottom-up and top-down forces and channeled energy flows from the benthic to the pelagic system (Kotta et al. in prep).

i) Recommendations

Based on prior studies the Harris mud crab was considered mostly omnivorous. The studies done in this BIO-C3 task show that in the more recently invaded areas (Northern Baltic Sea), they are mainly second-degree predators. Furthermore, they occupy habitats that are very valuable for the coastal ecosystems (*Fucus*-beds) and prey upon the grazers in these systems. Conversely, the native predators have adopted the novel item in their diet. Mud crabs are found in the stomachs of several fish, and most often in a generalist benthic predator, the four horned sculpin.

The project provided crucial information on basic ecological interactions of this novel species in their newly invaded areas and provides a background for new research projects, such as food web modelling.

i) List of publications:

Aarnio, K., A. Törnroos, C. Björklund & Bonsdorff, E., 2015: Food web positioning of a recent coloniser: the North American Harris mud crab *Rhithropanopeus harrisii* (Gould, 1841) in the northern Baltic Sea. - Aquatic Invasions 10: 399-413. doi.org/10.3391/ai.2015.10.4.04 (P13)

Kotta, J., Wernberg, T., Jänes, H., Kotta, I., Nurkse, K., Pärnoja, M., Orav-Kotta, H. (in prep): Introduction of a new ecological function causes marine ecosystem regime shift. (P6)

Nurkse, K., Kotta, J., Orav-Kotta, H., Pärnoja, M. & Kuprijanov, I. 2015: Laboratory experiment on the habitat occupancy of the crab *Rhithropanopeus harrisii* (Gould) in an invaded ecosystem: The north-eastern Baltic Sea. Estuarine Coastal and Shelf Science, 154, 152
doi:10.1016/j.ecss.2014.12.046. (P6)

Ovaskainen, A. 2015: Mud crab in the diet of fish and its distribution in the Archipelago Sea. Liejutaskurapu (*Rhithropanopeus harrisii*) kalojen ravinnossa ja sen levinneisyys Saaristomerellä. Bachelor's thesis. Turku AMK. In Finnish. 76pp. (P7)

Puntila R., Fowler A., Riipinen K., Vesakoski O. & Lehtiniemi M. (in prep a): Invasive Harris mud crab (*Rhithropanopeus harrisii*) prefers isopod prey in the Northern Baltic Sea. (P7)

Puntila, R., Loisa, O., Fowler, A. & Riipinen, K. (in prep b): A taste for aliens? Incorporation of a novel prey item into native fishes diet. (P7)

III.3. Impacts of the Grass prawn, *Palaemon elegans*

iii) Abstract /highlights

The Grass prawn *Palaemon elegans* has been colonizing the Baltic Sea since early 2000's. In the southern Baltic their success may have contributed to a decline of the native shrimp *P. adspersus*, but similar pattern has not been observed in the northern Baltic Sea range. In

addition, based on field samplings in the north both species are abundant in similar environments and no impacts were detected. Furthermore, in feeding experiments they show very similar responses. However, the non-native *P. elegans* shows more aggression towards *P. adspersus* than vice versa under experimental conditions. If competition will be more intense in the future, it may arise from behavioural differences. The role of *P. elegans* in the food web was also studied and results were somewhat conflicting. In Lithuania the species is a frequent prey for perch and cod whereas in Finland only two perch caught from a location where shrimp were abundant had shrimp remains in their stomachs. Furthermore, the abundance of the shrimp varies seasonally and between years. It seems that perch may need more time to adjust on feeding on this novel prey.

ix) Progress and (if applicable) deviations from the original work-plan

All the work has been completed, material has been largely analysed and the work either in manuscripts or already published.

x) Deviations from the work-plan

None.

xi) Introduction

Grass prawn (or rockpool prawn) *Palaemon elegans* (Rathke 1837) is an omnivorous littoral invertebrate that has become the dominating prawn in many locations the Baltic Sea. Its native range extends from the Black Sea to the Mediterranean Sea and to the Atlantic east coast from Scotland and Norway to Mauritania (d'Udekem d'Acoz 1999, cited in Reuschel et al. 2010). The first observations in the Baltic Sea were in 2002 from Arkona Basin (Zettler 2003) and the Gulf of Gdansk (Janas et al. 2004), although it had been sporadically detected in the German coast (Wismar Bay) already prior to these observations (e.g., Berglund 1985). Reuschel et al. (2010), showed that specimens collected from the SE Baltic Sea belong to the same haplotype group than the populations from the Black, Caspian and Mediterranean Seas, and are not similar to those from the Atlantic Ocean. This is implying significant human assistance in the introduction and further spread in the Baltic Sea.

Following the initial observations, *P. elegans* began colonizing shores along the southern and south-western coasts of the Baltic Sea rather rapidly (Grabowski 2006) and expanded its range to SE Baltic coasts (Daunys and Zettler 2006). In 2002 *P. elegans* was detected in Lithuania (E. Bacevičius personal communication), in Finland in 2003 and 2011 in Estonia (Kotta & Kuprijanov 2012).

P. elegans has caused changes in the native ecosystem possibly reducing infaunal and epifaunal density through selective feeding (Janas and Baranska 2008). Their increase has also lead to decline in the native *P. adspersus* populations (Grabowski 2006). Rapid increase in abundance, observed in the recent years in the coasts of Lithuania, Estonia and Finland, can have similar consequences.

xii) Methods and results

Feeding ecology and interactions of the native *Palaemon adspersus* and the invasive Grass prawn *Palaemon elegans*.

Macroalgae are an important habitat for small mobile invertebrates such as gammarid amphipods and palaemonid prawns. Gammarid amphipods are important grazers of micro- and

macroalgae whereas palaemonid prawns are feeding on macroalgae and small aquatic invertebrates including gammarids. As *P. elegans* occurs within the same habitats as the native *P. adspersus*, it is expected that this invasion modifies the existing trophic interactions. To address this question, the feeding of the native *P. adspersus* and the invasive *P. elegans* on the benthic macroalga *Cladophora glomerata* and on the invasive gammarid amphipod *Gammarus tigrinus* was experimentally investigated by UT-EMI (P6). The results showed that the alien prawn does not add an extra function to the trophic system of the coastal ecosystem of the Baltic Sea (Kuprijanov et al. 2015). Nevertheless, due to its progressively increasing densities and wide habitat range, *P. elegans* is expected to exert stronger predation pressure on gammarid amphipods as compared to *P. adspersus* alone.

The impacts of *Palaemon elegans* were investigated in coastal areas of the northern Baltic Sea by SYKE (P7). Associated fauna of both *Fucus* and *Phragmites* habitats were sampled through the summer months in several locations with varying shrimp abundances (both native *P. adspersus* and non-native *P. elegans*). The samples were obtained by snorkeling with hand nets and using small traps. The results show that the species appear to co-exist and are both numerous in similar habitats. It also appears that non-native *P. elegans* is not, at least currently, competitively excluding the native *P. adspersus* or significantly altering the grazer fauna in the area (Puntilla et al. in prep). This is likely due to the very similar ecology of these two species. Furthermore, the prevalence of *P. elegans* in perch (*Perca fluviatilis*) stomachs was studied in the same areas as above (Aalto, M. BSc thesis). Altogether 90 fish stomachs were collected from sites where shrimps were numerous in 2013 (May, July and September). The stomach contents from perch ranging from 100 to 350 mm TL were carefully inspected for shrimp remains. The results showed that perch do not seem to consume shrimps in our sampling areas, since *Palaemon* shrimp remains were detected in only two stomachs (TL 219 mm and 205 mm). The species could not be identified from the remains. Furthermore, the stomach analyses from more than 1300 fish from the Archipelago Sea were inspected for the remains of *P. elegans*. Again, they were detected only from few fish (Ovaskainen 2015). This implies limited predation from fish in the coastal areas of Finland, northern Baltic Sea.

The behavioral differences of the native *P. adspersus* and non-native *P. elegans* were studied experimentally by SYKE (P7) (Korhonen, A. MSc thesis). The shrimps were placed in aquaria either by themselves, with an individual of their own species or with an individual from the other species. The experiments were video recorded and shrimp behavior was then evaluated from the recordings. Prior to the experiments the shrimps were labelled by feeding them either green or red food, making them easy to distinguish from the recordings. Results show that the individual variation in behavior is large in both species. Furthermore, *P. elegans* showed more aggression when combined with the native *P. adspersus* than vice versa and when combined with another *P. elegans*. When predator (*Perca fluviatilis*) was visible, aggressive behavior increased in both species. This may contribute to greater fitness of *P. elegans*, especially in low predation risk environments (see above).

Effect of invasive species on community trophic structure, exploring new trophic links and native species niche shifts due to invader effects: the model species - grass prawn *Palaemon elegans*.

The seasonal abundance dynamic of the non-indigenous prawn *Palaemon elegans* was studied by KU-CORPI (P8) in shallow (0.5–1.6 m) stony habitat of the exposed coast of the SE Baltic Sea in 2014 and 2015. 4 replicate semi-quantitative samples were collected using 25×25cm hand net in 1-3 weeks intervals. The area is influenced by a eutrophic water plume from the Curonian

Lagoon. Mean salinity is 6.0 ± 1.2 and chlorophyll *a* concentrations vary from 0.7 to 156 mg m^{-3} . We found that in this shallow habitat prawn abundance is highly related to reproductive cycle. The maximum numbers were observed during the spawning periods July 16, 2014 $504 \pm 265 \text{ ind. m}^{-2}$ and August 11, 2015 $704 \pm 299 \text{ ind. m}^{-2}$. At that time population was dominated by egg carrying females. The first individuals of the new generation occurred on the July 31 and September 14 in 2014 and 2015, respectively. One month difference between the year's is most probably related to slower egg and larval development rates at lower temperature in the summer 2015 ($<20^\circ\text{C}$) (Fig. 11, Vaitkute, 2017).

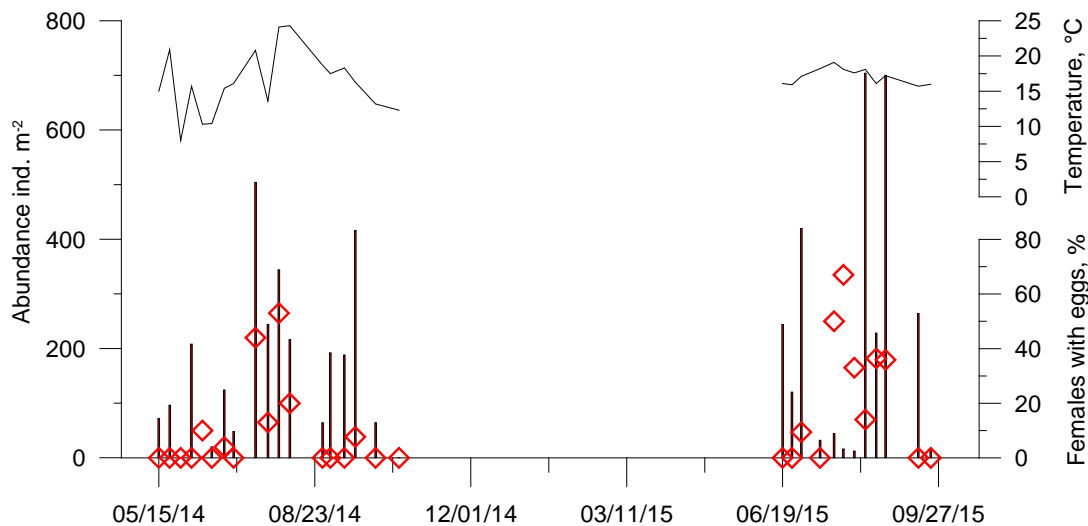


Figure 11. The seasonal abundance dynamic of *Palaemon elegans* in the artificial stony habitat (harbor mole) of the exposed coast of the SE Baltic Sea in 2014 and 2015. The maximum share of fertile females (%) is indicated by diamonds.

The share of *P. elegans* in the diet of predatory fish: perch (*Perca fluviatilis*) and cod (*Gadus morhua*) was also studied by KU-CORPI (P8) (Skabeikis 2017). For diet analysis fish were collected in the area of stony bottom using gill nets at the depth ranging from 5 to 12 m. Perch was collected in July, while cod in October-November, 2014. *P. elegans* occurred in 47% of dissected stomachs of smaller perch ($L = 12 \pm 0.8 \text{ cm}$) and 41% of larger individuals ($L = 19 \pm 2.4 \text{ cm}$). The average share in the diet was 43% and 22% in the small and larger perch, respectively (Fig. 8). Although *P. elegans* disappears from the shallow ($<1.5\text{m}$) habitats in the autumn it is found in 28% of cod ($L = 34 \pm 5 \text{ cm}$) stomachs collected at that time at higher depth. It's share in the cod diet on average was 24%, while *Neogobius melanostomus* contributed $\sim 40\%$.

We can state that in the SE Baltic Sea invasive species *P. elegans* and *N. melanostomus* become important food components of local predators. Therefore we can expect some top-down regulating effects of the invader populations in the future. This way *P. elegans* provides a new pathway from primary producers (phytoplankton and macroalgae) to higher trophic levels. The intraguild predation on mysids and amphipods should be however taken in to account because these groups are important components of autochthonous communities in the Baltic Sea and also serve as food items for young fish.

i) Recommendations

The significant changes in the coastal vegetated habitats as a result of the invasion by non-native *P. elegans* are yet to be confirmed in the northern Baltic Sea. The potential reason lies behind

the ecological similarity to the native shrimp *P. adspersus*. However, experimental work shows that these species have subtle differences in their behaviour and feeding that may translate to more pronounced impacts in the future if population densities increase.

Furthermore, the prawn is a frequent prey to fishes in Lithuania by contributing significantly to perch and cod diets. This implies to at least some potential for predation control over this invasive species. Conversely, in Finland perch do not seem to prey upon them. Thus regional differences in top-down control are clear and understanding the mechanisms affecting these interactions require more studies.

i) List of publications:

Aalto, M., BSc thesis (in prep): The invasive Grass prawn (*Palaemon elegans*) in the diet of predatory fish in the Gulf of Finland. Vieraslaji sirokatkarapu (*Palaemon elegans*) petokalojen ravintokohteena Suomenlahdella. In Finnish. (P7)

Korhonen, A., Master's thesis (in prep): The battle of the prawns – interactions between the invasive Grass prawn (*Palaemon elegans*) and the native *Palaemon adspersus*. Katkarapujen taisto – Vieraslaji sirokatkaravun (*Palaemon elegans*) ja alkuperäislaji leväkatkaravun (*P. adspersus*) väliset vuorovaikutukset. In Finnish. (P7)

Kuprijanov, I., Kotta, J., Lauringson, V., & Herkül, K. (2015). Trophic interactions between native and alien palaemonid prawns and an alien gammarid in a brackish water ecosystem. Proceedings of the Estonian Academy of Sciences, 64(4), 518-524. (P6)

Puntila, R. et al. (in prep): The abundance and impacts of non-native shrimp *Palaemon elegans* in coastal vegetated habitats in the Gulf of Finland. (P7)

Skabeikis A. PhD theses (in prep): The role of round goby (*Neogobius melanostomus*) in the food web of SE Baltic Sea open coast food web. (P8)

Vaitkute K., BS thesis (in prep): The reproduction and population dynamic of invasive prawn *Palaemon elegans*. Invazinės krevetės *Palaemon elegans* reprodukcijos dėsningumai ir gausumo sezoninė dinamika. In Lithuanian. (P8)

III.4. Impacts of the invasive polychaetes, *Marenzelleria* spp.

iv) Abstract /highlights

The invasive *Marenzelleria* spp. polychaetes are among the few invaders potentially impacting the Baltic Sea positively. They have an ability to burrow much deeper into the sediments than native species and can therefore oxygenate sediments and impacting also biogeochemical processes such as nutrient cycling. In the Central Baltic, in the Vistula Lagoon, Polish coast they have begun to dominate the benthos and significantly altered the benthic habitats. Species distribution modelling indicates that the abundance of *Marenzelleria* spp. in shallow water sediments makes them potential prey for coastal fish and in these areas competition with native species is likely. Furthermore, they have potential to increase phosphorus retention in bottom deposits due to deeper oxygen penetration into sediments and formation of a deeper oxidized layer. This may contribute to significant changes in nutrient cycling.

xiii) Progress and (if applicable) deviations from the original work-plan

Analyses have been completed and most of the results published.

xiv) Deviations from the work-plan

None.

xv) Introduction

Marenzelleria spp. Mesnil, 1896 are among the most successful non-native benthic species in the Baltic Sea. The group of species originates mostly from the Atlantic North American coastal estuaries (George 1966) and one of the species, *Marenzelleria arctia* (Chamberlin, 1920), from the Arctic (Bastrop and Blank 2006).

The species complex has been detected in the Baltic Sea since 1985, when *Marenzelleria neglecta* was recorded in the German Darb-Zingst bodden chain (Bick and Burkhardt 1989). By mid-1990's the species was present in most of the Baltic Sea and currently they are abundant throughout the area (Kauppi et al. 2015). Originally the species were identified as *M. viridis* (Verill), but following a revision of the genus, they were reassigned as *M. neglecta* (Sikorski and Bick, 2004). However, molecular methods imply that the Baltic Sea is currently actively colonized by several morphologically very similar species *Marenzelleria*. This taxonomic confusion has complicated the research of the impacts of the species complex in the Baltic Sea.

Marenzelleria spp. are considered among the most successful NIS recently introduced to the Baltic Sea (Zettler et al., 2002). These polychaetes burrow into sediment at depths of up to 40 cm (Zmudzinski 1996), which is 10–35 cm deeper than the indigenous Baltic Sea infauna is capable for (Rumorhr et al. 1996, Bradshaw et al. 2006). The local impacts of the genus has been previously studied in some areas (e.g., Kotta et al. 2001, 2003, Granberg et al. 2008), but information was lacking on the impacts and population structure of the genus in some areas (Poland and Lithuania), their role in the food web as well as their potential impacts on the nutrient cycling. The project's results aim at answering these questions.

xvi) Methods and results

Population dynamics and spread of *Marenzelleria* spp. and other benthic invasive species in the Polish coast

All available data on introductions of non-indigenous species to the Vistula Lagoon, from the 19th onwards, were compiled and analysed to assess the impact of those species on the structural and functional diversity of the Lagoon's benthos by NMFRI (P5) (Warzocha J, in prep.).

The Vistula Lagoon is a shallow brackish water body connected with the Baltic Sea via a strait. In the analyses, non-indigenous species inhabiting the inshore rooted vegetation (primarily reed belts) outside the waterline were disregarded.

In the 19th century, large inputs of fresh water supplied with the River Vistula discharge was the major factor shaping life conditions of the macrobenthos. The macrozoobenthos consisted primarily of freshwater oligochaetes and chironomid larvae. Suspension feeders were practically absent. In the early 20th century, a canal guiding the Vistula discharge directly to the Baltic Sea was dredged, and sluices which substantially reduced the freshwater supply to the Lagoon were constructed. Therefore, seawater incursions into the Lagoon via the strait became the major factor affecting environmental conditions. The salinity increased in the western (Polish) part of the Lagoon from about 0.5 to about 3.

Paradoxically, however, the first non-indigenous species to settle in large numbers on the bottom in the western part of the Lagoon was the zebra mussel *Dreissena polymorpha*, a freshwater bivalve. Bivalves had been absent from the sublittoral bottom, and the zebra mussel was the first suspension feeder in the Lagoon. Its population developed very dynamically, but the mass occurrence was restricted to areas located away from the strait, i.e. showing a relatively low salinity.

In addition to riverine discharge regulation, another important factor affecting the environmental conditions in the Lagoon was the eutrophication, observed as of the 1960s. The associated environmental changes, in particular the transformation of the sandy-muddy bottom into a muddy one, and the likely oxygen depletion, resulted in almost complete elimination of the zebra mussel and restriction of its occurrence to submerged vegetation.

In the late 1980s, the entire bottom of the Lagoon, both in its sandy and muddy part, was colonised by the polychaete *Marenzelleria*. The polychaete, both a detritivore and a suspension feeder, substantially altered the food web in the Lagoon's ecosystem. The abundance and biomass soared to 8000 ind m⁻² and more than 400 g ww m⁻². Prior to the introduction of *Marenzelleria*, the total macrozoobenthos biomass did not exceed a few to several grams wet weight per m². There are no data on the extent of the polychaete being used by fish as food.

Another new immigrant which has been observed to very dynamically spread in the Lagoon since about 2010 and which has colonised the entire area is a bivalve *Rangia cuneata*. Despite extensive fluctuations in the spatial distribution, abundance and biomass, the abundance reaches several tens of individuals per m² bottom area, the biomass even exceeding 150 g m⁻². A drastic reduction of the abundance was observed when the ice cover persisted for periods of time longer than usual. However, in the spring, the bivalve's pelagic larvae re-colonised the entire Lagoon.

The bivalve has substantially increased the suspension feeders' contribution to the macrozoobenthos of the Lagoon (Fig. 12). Preliminary studies ruled out fish feeding on the bivalve. There are no reliable data, either, on effects of the changes in the macrozoobenthos trophic structure on the ecosystem functioning in the Polish part of the Vistula Lagoon.

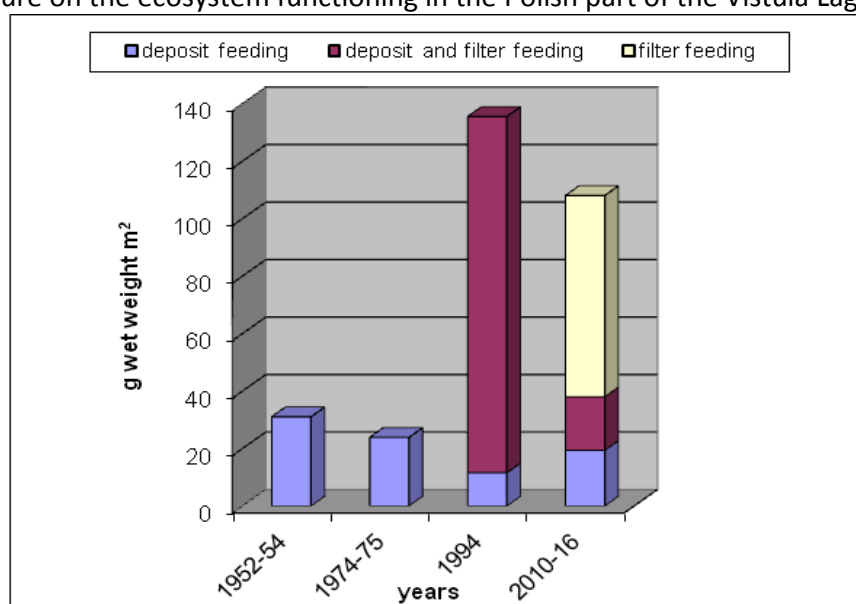


Fig. 12. Long-term changes in the trophic structure of macrozoobenthos in the Vistula Lagoon (muddy bottom).

Contribution of the invasive polychaete *Marenzelleria neglecta* to fish diet and the quality of benthophagous fish feeding grounds (empirical modelling, fish feeding grounds mapping)

Species distribution modelling of *Marenzelleria* complex was performed for the spatial information on the biomass in Lithuanian marine area by KU-CORPI (P8) (contribution from Task 4.1). Also, the share of *Marenzelleria* spp. in local benthic communities was estimated. Data on the occurrence and importance in the fish diet were collected for Baltic cod, flounder and eelpout (Šiaulyš 2015).

The model showed that areas of the highest biomass is located in shallow and exposed sandy bottoms (3-10 m depth), where species richness is relatively low (3-5 species). In these habitats spionid polychaetes can comprise up to 50 % of biomass in the zoobenthic community. It is yet to be confirmed whether *Marenzelleria* spp. outcompeted local species, such as other polychaete *Hediste diversicolor* or occupied empty niche thus increasing total biomass of zoobenthos. If the latter is true, this would suggest that the invasion of this species could have positive impact on mobile sand habitat, known as nursery areas of several fish species. In deeper areas the biomass share of *Marenzelleria* spp. in benthic community becomes relatively low, thus despite being a prey item for cod, flounder and eelpout, the importance for the diet of these fish species is low. The highest biomass of a non-indigenous spionid *Marenzelleria* spp. was observed in the shallow coastal areas, where local species richness is the lowest due to natural roughness of mobile sand habitat.

Impacts of the non-native *Marenzelleria* spp. on biogeochemical processes

The impacts of *Marenzelleria* spp. were studied by ÅA (P13) also from a biogeochemical point of view (Maximov et al. 2015). The species unique burrowing behaviour (they dig deeper than most native Baltic species) results in previously lacking ecosystem functions. They examined evidence from experiments, field sampling and modelling about the introduction of *Marenzelleria* spp. effects on nutrient cycling and biogeochemical processes at the sediment—water interface. Over longer time scales, bioirrigation by *Marenzelleria* spp. has the potential to increase phosphorus retention in bottom deposits because of deeper oxygen penetration into sediments and formation of a deeper oxidized layer. In contrast, nitrogen fluxes from the sediment increase. As a consequence of a decline of the phosphate concentration and/or rising nitrogen/phosphorus ratio, some Northern Baltic ecosystems may experience improvement of the environment because of mitigation of eutrophication and harmful cyanobacteria blooms. Although it is difficult to unambiguously estimate the ecosystem-level consequences of invasion, in many cases it could be considered as positive due to increased structural and functional diversity (Fig. 13). The long-term interactions with the native fauna still remain unknown.

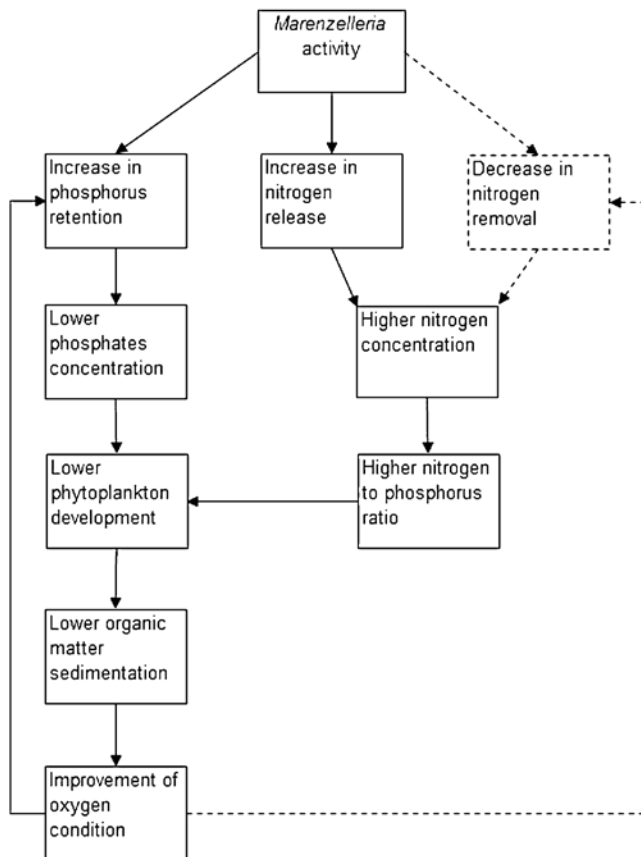


Figure 13. Potential effects of *Marenzelleria spp.* on nutrient cycling in the ecosystems of the Northern Baltic Sea. Dashed box and lines indicate a hypothesized process and effects with little data at this time (Maximov et al. 2015).

ii) Recommendations

Based on the results it appears that *Marenzelleria spp.* has caused a significant change in the Baltic Sea. Due to their unique ability to burrow deeper than native species, they are able to alter the physical characteristics of the sediment and impact the nutrient cycling. From this point of view their impacts can be considered “positive”. However, in other areas they appear to have colonized areas where they may compete with native species. Furthermore, in areas where they are abundant, they can become a frequent component in fishes diet.

i) References

Raišuočienė G. BS thesis (2016). The dynamics of the invasive polychaete *Marenzelleria sp.* (Mesnil 1986) and its relationship with the local zoobenthic community in the Lithuanian coastal area. Invazinės daugiašerės kirmėlės *Marenzelleria sp.* (Mesnil 1986) dinamika ir santykis su vietine zoobentosos bendrija Lietuvos priekrantėje. In Lithuanian. (P8)

Warzocha J. (in preparation) Impact of non-indigenous macrobenthic species on structural and functional diversity in the Vistula Lagoon (Southern Baltic Sea). (P5)

III.1. Invasive comb jelly *Mnemiopsis leidyi*

ii) Abstract /highlights

The non-indigenous comb jelly *Mnemiopsis leidyi* has been present in Northern Europe since 2005 and reaches invasive status in several regions such as the Kattegat, Limfjorden and the SW North Sea (Jaspers, Huwer et al. (a) in review). For Northern Europe, we show for the first time that low winter temperatures have a significant effect on the range expansion of *M. leidyi* and re-seeding of animals from high abundance hot-spots has been documented to be extremely fast with more than 2,000 km year⁻¹ (Jaspers, Huwer et al. (a) in review). Also, we show that *M. leidyi* in invasive sub-populations show selection for traits to optimize its population growth, compared to native sub-populations which optimize for individual life time reproductive success (Jaspers et al. (b) in review). Combined, these two major findings show that *M. leidyi* is a very potent invasive species due to its fast population growth but also its swift re-colonization following local extinctions. Partner P2, P1, P11 wrote this section and contributions from all partners are outlined below:

iii) Progress and (if applicable) deviations from the original work-plan

We compiled a database of 12,000 presence/absence data of *Mnemiopsis leidyi* throughout western Eurasia. The manuscript is in review. A common garden experiment with northern and southern invasive sub-populations has been conducted in 2015. Those data contribute to a manuscript (b) which is in review. Additionally, a M.Sc. thesis has investigated larval reproduction on population level in different invasive sub-populations.

iv) Deviations from the work-plan

None

v) Introduction

The non-indigenous comb jelly *Mnemiopsis leidyi* stems from the east coast of Americas and has been present in western Eurasia since the 1980's (as reviewed in Jaspers, Huwer et al. (a)). Two different invasion events can be differentiated with the first one into the Black Sea in the mid 1980's and an independent second introduction into northern European waters in the mid 2000's (Reusch et al. 2010).

The sea walnut has been present in the extended Baltic Sea region since 2005, while animals have first been recorded in the Baltic Sea during summer 2006 (Kube et al. 2007, as reviewed in Jaspers, Huwer et al. (a)). In situ reproduction experiments in different salinity regions of the Baltic Sea, as well as laboratory controlled egg production experiments revealed, that *M. leidyi* cannot sustain populations in the low saline central and north-eastern Baltic Sea (Jaspers et al. 2011, Lehtiniemi et al., 2011). Adaptation potential to circumvent these current constrains has been considered and is reported in WP 1.3.

vi) Methods and results

This study is the first of its kind to look at continental connectivity and invasion corridors in the marine realm. The results of the here presented synthesis (Jaspers, Huwer et al. (a) in review - P1, P2, P3, P5, P7) documents the importance of ocean currents for bio-invasions. We used the American comb jelly *Mnemiopsis leidyi* as a biological probe, representing invasive holoplanktonic species in general. Though ballast water is believed as initial and secondary invasion vector, we show that secondary introductions within western Eurasia are driven by ocean currents and can as such explain observed invasion corridors. North European regional extinctions after cold winters were followed by re-colonization of new genotypes with a velocity of up to 2,000 km year⁻¹. This provides a natural experimental proof for ocean currents driving metapopulation dynamics. Our results demonstrate that hot-spots hosting invasives in highly inter-connected areas are key for secondary spread dynamics of holoplanktonic species on a

continental scale. This points towards the importance of ocean current connectivity for sustaining non-indigenous species in less favourable habitats, such as the low saline central and northern Baltic Sea.

For our biological probe species, in higher saline areas, such as the Kattegat and Skagerrak, negative impacts on food web structure and functioning have lately been documented (e.g. Tiselius and Møller 2017). Due to its higher reproduction potential in higher saline regions, and basically non-reproduction under central Baltic Sea salinities (Jaspers et al. 2011), these higher saline Baltic Sea areas are suggested as potential seed region maintaining populations in the low saline central Baltic Sea on an annual basis. A manuscript is under preparation where we show that such high saline areas of the Baltic Sea can function as seed region for sustaining low saline sink populations in the central Baltic Sea on an annual basis (Jaspers, Hinrichsen et al. in preparation). However, irrespectively of intensive monitoring activities conducted within the BIO-C3 consortium, *M. leidy* has not been confirmed further to the North East of the Baltic Sea than the Bornholm Basin/South Eastern Gotland Basin. All data are compiled in a database and part the synthesis paper (Jaspers, Huwer et al. (a) in review) (Fig. 13). The data will be deposited on dryad upon acceptance of the manuscript.

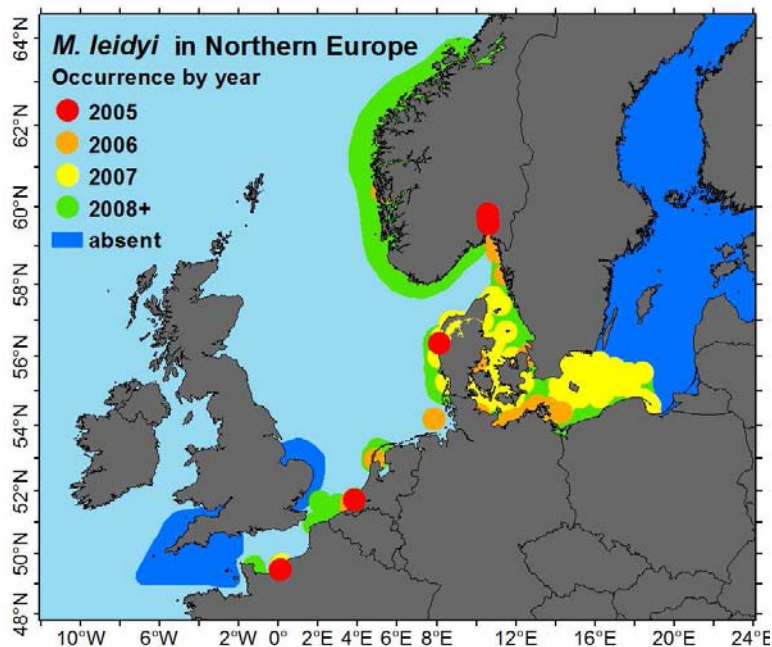


Figure 13. Range expansion of *Mnemiopsis leidy* following the first 3 years after recognition of this invasive comb jelly in northern Europe (part of Jaspers et al. (a) in review, P1, P2, P3, P5, P7).

Food web interactions: Additionally, a manuscript on the predation impact on herring larvae within Greifswald Bay is under preparation (Kotterba et al., submitted as supplement to Deliverable report 2.2). Although native species were expected to be the main predators on herring larvae, we also investigated the spatio-temporal overlap of herring larvae with the invasive ctenophore *Mnemiopsis leidy*. Although the *M. leidy* abundances can be notably high in Greifswald Bay during summer, they do not co-occur with herring larvae, which peak during spring (April-May).

vii) Recommendations

Ballast water release into highly inter-connected areas is of major concern since non-indigenous species can be transported over large areas (Jaspers et al. (a) – in review). In case of the

American comb jelly *Mnemiopsis leidyi*, we show that low winter temperatures in combination with ocean current connectivity set the scene for their range occupancy in non-native habitats. Highly inter-connected hot-spot areas of the comb jelly *M. leidyi*, as outlined in Jaspers et al. (a) are important for maintaining populations in northern Europe and especially the Baltic Sea.

Since *M. leidyi* forms hot spot areas in highly inter-connected areas, it is a lost battle to prevent re-current re-invasions. So far low saline areas of the Baltic Sea are dependent on drift recruitment from higher saline areas (Jaspers, Hinrichsen et al. in preparation). But introduction of new genotype into North European populations with the potential to circumvent current constraints on its distribution should be prevented by all means. This is especially so since latest results document that >99% of the offspring of *M. leidyi* is outbred, hence cross-fertilization is favoured over self-fertilization in this basal metazoan (Jaspers & Reusch in preparation). Therefore, results are highly relevant for the new ballast water convention to point towards high risk areas where ballast water release should be prevented by all means without any exceptions.

As a final remark, we would like to highlight the fruitful, inter-disciplinary work which has been carried out as part of the BIO-C3 consortium. However, there are a lot of unknowns which should be considered for future work such as intensive monitoring activities considering genotypic change over time for key non-indigenous species.

viii) References

Jaspers, C., Huwer, B., Antajan, E., Hosia, A., Hinrichsen, H.-H., Biastoch, A., Angel, D., Asmus, R., Augustin, C., Bagheri, S., Beggs, S.E., Balsby, T.J.S., Boersma, M., Bonnet, D., Christensen, J.T., Daenhardt, A., Delpy, F., Falkenhaus, T., Finenko, G., Fleming, N., Fuentes, V., Galil, B., Griffin, D.C., Haslob, H., Javidpour, J., Kamburska, L., Kube, S., Langenberg, V., Lehtiniemi, M., Lombard, F., Malzahn, A., Marambio, M., Mihneva, V., Møller, L.F., Niermann, U., Okyar, M.I., Özdemir, Z.B., Pitois, S., Reusch, T.B.H., Robbens, J., Stefanova, K., Thibault, D., van der Veer, H., Vansteenbrugge, L., van Walraven, L., Woźniczka, A. (a - in review) Ocean current connectivity sets the scene for secondary spread of a marine invader across western Eurasia.

Jaspers, C., Marty, L. and Kjørboe, T. (b – in review) Selection for life-history traits to maximize population growth in an invasive marine species.

Jaspers, C., Reusch, T.B.H., Huwer, B., Hinrichsen, H.-H. (in preparation) Hot-spots sustaining high abundances of invasive species can act as accelerator allowing for re-colonization after local extinctions of less favorable habitats.

Jaspers, C. and Reusch, T.B.H. (in preparation) The invasive comb jelly *Mnemiopsis leidyi* prevents self-fertilization to maintain high standing genetic variation.

Kaehlert S., Bading K., Javidpour J., Jaspers C. (manuscript to be submitted) Ctenophores are early birds – a study on larval reproduction of the American comb jelly *Mnemiopsis leidyi*.

Kotterba et al. (manuscript as attached to deliverable report 2.2 during Jan 2017)

IV. References of the report

- Aarnio, K., A. Törnroos, C. Björklund, and E. Bonsdorff. 2015. Food web positioning of a recent coloniser: the North American Harris mud crab *Rhithropanopeus harrisi* (Gould, 1841) in the northern Baltic Sea. *Aquatic Invasions* 10:399–413.
- Almqvist, G., A. K. Strandmark, and M. Appelberg. 2010. Has the invasive round goby caused new links in Baltic food webs? *Environmental Biology of Fishes* 89:79–93.
- Bacevicius, E., and Z. R. Gasiunaite. 2008. Two crab species-Chinese mitten crab (*Eriocheir sinensis* Edw.) and mud crab (*Rhithropanopeus harrisi* (Gould) ssp. *tridentatus* (Maitland)) in the Lithuanian coastal waters, Baltic Sea. *Transitional Waters Bulletin* 2:63–68.
- Balshine, S., A. Verma, V. Chant, and T. Theysmeyer. 2005. Competitive interactions between round gobies and logperch. *Journal of Great Lakes Research* 31:68–77.
- Bastrop, R., and M. Blank. 2006. Multiple Invasions – A Polychaete Genus Enters the Baltic Sea. *Biological Invasions* 8:1195–1200.
- Berglund, A. 1985. Different Reproductive Success at Low Salinity Determines the Estuarine Distribution of two *Palaemon* Prawn Species. *Holarctic Ecology* 8:49–52.
- Bick, A., and R. Burckhardt. 1989. Erstnachweis von *Marenzelleria viridis* (Polychaeta, Spionidae) für den Ostseeraum, mit einem Bestimmungsschlüssel der Spioniden der Ostsee. *Zoosystematics and evolution* 65:237–247.
- Bradshaw, C., Kumblad, L., Fagrell, A. 2006: The use of tracers to evaluate the importance of bioturbation in remobilising contaminants in Baltic sediments. *Estuarine, Coastal Shelf Sci.*, 66, 123–134.
- Charlebois, P. M., J. E. Marsden, R. G. Goettel, R. K. Wolfe, D. J. Jude, and S. Rudnika. 1997. The Round Goby *Neogobius melanostomus* (Pallas) A Review of European and North American Literature. Illinois-Indiana Sea Grant Program and Illinois Natural History Survey. INHS Special Publication No. 20.
- Czerniejewski, P., and A. Rybczyk. 2008. Body weight, morphometry, and diet of the mud crab, *Rhithropanopeus harrisi tridentatus* (Maitland, 1874) in the Odra estuary, Poland. *Crustaceana* 81:1289–1299.
- Daunys, D., and M. L. Zettler. 2006. Invasion of the North American Amphipod (*Gammarus tigrinus* Sexton, 1939) into the Curonian Lagoon, South-Eastern Baltic Sea. *Acta Zoologica Lituonica* 16:20–26.
- Demel, K. 1953. Nowy gatunek w faunie Bałtyku. *Kosmos* 2:105–106.
- Dietrich, J. P., B. J. Morrison, and J. A. Hoyle. 2006. Alternative Ecological Pathways in the Eastern Lake Ontario Food Web — Round Goby in the Diet of Lake Trout. *Journal of Great Lakes Research* 32:395–400.
- Dubs, D. O. L., and L. D. Corkum. 1996. Behavioral Interactions Between Round Gobies (*Neogobius melanostomus*) and Mottled Sculpins (*Cottus bairdi*). *Journal of Great Lakes Research* 22:838–844.
- Forsström, T., A. E. Fowler, I. Manninen, and O. Vesakoski. 2015. An introduced species meets the local fauna: predatory behavior of the crab *Rhithropanopeus harrisi* in the Northern Baltic Sea. *Biological Invasions* 17:2729–2741.

- Fowler, A., T. Forsström, M. von Numers, and O. Vesakoski. 2013. The North American mud crab *Rhithropanopeus harrisi* (Gould, 1841) in newly colonized Northern Baltic Sea: distribution and ecology. *Aquatic Invasions* 8:89–96.
- George, J. D. 1966. Reproduction and early development of the spionid polychaete, *Scolecopides viridis* (Verrill). *Biol. Bull., Mar. Biol. Lab., Woods Hole* 130:76–93.
- Grabowski, M. 2006. Rapid colonization of the Polish Baltic coast by an Atlantic palaemonid shrimp *Palaemon elegans* Rathke, 1837. *Aquatic Invasions* 1:116–123.
- Granberg, M. E., J. S. Gunnarsson, J. E. Hedman, R. Rosenberg, and P. Jonsson. 2008. Bioturbation-driven release of organic contaminants from Baltic Sea sediments mediated by the invading polychaete *Marenzelleria neglecta*. *Environmental Science and Technology* 42:1058–1065.
- Hegele-Drywa, J., and M. Normant. 2009. Feeding ecology of the American crab *Rhithropanopeus harrisi* (Crustacea, Decapoda) in the coastal waters of the Baltic Sea. *Oceanologia* 51:361–375.
- Hines, A. H., F. Alvarez, and S. A. Reed. 1997. Introduced and native populations of a marine parasitic castrator: Variation in prevalence of the rhizocephalan *Loxothylacus panopaei* in xanthid crabs. *Bulletin of Marine Science* 61:197–214.
- Jakubas, D. 2004. The Response of the Grey Heron to a Rapid Increase of the Round Goby. *Waterbirds* 27:304–307.
- Janas, U., and A. Baranska. 2008. What is the diet of *Palaemon elegans* a non-indigenous species in the Gulf of Gdańsk (southern Baltic Sea)? *Oceanologia* 50:221–237.
- Janas, U., T. Zarzycki, and P. Kozik. 2004. *Palaemon elegans* - A new component of the Gulf of Gdańsk macrofauna. *Oceanologia* 46:143–146.
- Jaspers C., Møller, L. F. and T. Kiørboe. 2011 Salinity gradient of the Baltic Sea limits the reproduction and population expansion of the newly invaded comb jelly *Mnemiopsis leidyi*. *PLoS One* 6:e24065-e24065.
- Järv, L., J. Kotta, I. Kotta, and T. Raid. 2011. Linking the structure of benthic invertebrate communities and the diet of native and invasive fish species in a brackish water ecosystem. *Annales Zoologici Fennici* 2450:129–141.
- Jormalainen, V., K. Gagnon, and E. Rotha. 2016. The invasive mud crab enforces a major shift in a rocky littoral invertebrate community of the Baltic Sea. *Biological Invasions* in press.
- Karhilahti, A. 2010. Taskurapu tarttui pyydykseen. *Suomen luonto* 4. Suomen Luonto.
- Karlson, A. M. L., G. Almqvist, K. E. Skora, and M. Appelberg. 2007. Indications of competition between non-indigenous round goby and native flounder in the Baltic Sea. *Ices Journal of Marine Science* 64:479–486.
- Kauppi, L., A. Norkko, and J. Norkko. 2015. Large-scale species invasion into a low-diversity system: spatial and temporal distribution of the invasive polychaetes *Marenzelleria spp.* in the Baltic Sea. *Biological Invasions* 17(7): 2055–2074

- Kotta, J., and I. Kuprijanov. 2012. The first finding of the palaemonid shrimp *Palaemon elegans* Rathke in the Estonian coastal sea. *Estonian Journal of Ecology* 61:148–153.
- Kotta, J., K. Nurkse, R. Puntila, and H. Ojaveer. 2016. Shipping and natural environmental conditions determine the distribution of the invasive non-indigenous round goby *Neogobius melanostomus* in a regional sea. *Estuarine, Coastal and Shelf Science* 169:15–24.
- Kotta, J., and H. Ojaveer. 2012. Rapid establishment of the alien crab *Rhithropanopeus harrisi* (Gould) in the Gulf of Riga. *Estonian Journal of Ecology* 61:293.
- Kotta, J., and E. Ólafsson. 2003. Competition for food between the introduced polychaete *Marenzelleria viridis* (Verrill) and the native amphipod *Monoporeia affinis* Lindström in the Baltic Sea. *Journal of Sea Research* 50:27–35.
- Kotta, J., H. Orav, and E. Sandberg-Kilpi. 2001. Ecological consequence of the introduction of the polychaete *Marenzelleria cf. viridis* into a shallow-water biotope of the northern Baltic Sea. *Journal of Sea Research* 46:273–280.
- Kube S., Postel, L., Honnef, C. and C. B. Augustin. 2007. *Mnemiopsis leidyi* in the Baltic Sea – distribution and overwintering between autumn 2006 and spring 2007. *Aquatic Invasions* 2:137-145.
- Lauer, T. E., P. J. Allen, and T. S. McComish. 2004. Changes in mottled sculpin and johnny darter trawl catches after the appearance of round gobies in the Indiana waters of Lake Michigan. *Transactions of the American Fisheries Society* 133:185–189.
- Lederer, A., J. Massart, and J. Janssen. 2006. Impact of round gobies (*Neogobius melanostomus*) on dreissenids (*Dreissena polymorpha* and *Dreissena bugensis*) and the associated macroinvertebrate community across an invasion front. *Journal of Great Lakes Research* 32:1–10.
- Lee, V., Johnson, T., 2005: Development of a Bioenergetics Model for the round goby (*Neogobius melanostomus*) *J. Great Lakes Res.* 31:125–134
- Lehtiniemi M, Lehmann, A, Javidpour, J and K. Myrberg. 2011. Spreading and physico-biological reproduction limitations of the invasive American comb jelly *Mnemiopsis leidyi* in the Baltic Sea. *Biological Invasions*, 14, 341-354.
- Maximov, A., E. Bonsdorff, T. Eremina, L. Kauppi, A. Norkko & J. Norkko, 2015: Context-dependent consequences of *Marenzelleria* spp. (Spionidae: Polychaeta) invasion on nutrient cycling in the Northern Baltic Sea. - *Oceanologia* 57: 342-348, doi: 10.1016/j.oceano.2015.06.002
- Moskalkova, K. I. 1996. Ecological and morphophysiological prerequisites to range extension in the round goby *Neogobius melanostomus* under conditions of anthropogenic pollution. *Journal of Ichthyology* 36:584–590.
- Nurkse, K., J. Kotta, H. Orav-Kotta, M. Pärnoja, and I. Kuprijanov. 2015. Laboratory analysis of the habitat occupancy of the crab *Rhithropanopeus harrisi* (Gould) in an invaded ecosystem: The north-eastern Baltic Sea. *Estuarine, Coastal and Shelf Science* 154:152–157.
- Odum, W. E., and E. J. Heald. 1972. Trophic Analyses of an Estuarine Mangrove Community. *Bulletin of Marine Science* 22:671–738.

- Panov, V. E., Y. Y. Dgebuadze, A. Shiganova, A. A. Filippov, and D. Minchin. 2007. A risk assessment of biological invasions in the inland waterways of Europe: the Northern Invasion Corridor case study. Pages 639–656 in F. Gherardi, editor. Biological invaders in inland waters: Profiles, distribution, and threats. Springer Series in Invasion Ecology Vol 2.
- Raby, G. D., L. F. G. Gutowsky, and M. G. Fox. 2010. Diet composition and consumption rate in round goby (*Neogobius melanostomus*) in its expansion phase in the Trent River, Ontario. *Environmental Biology of Fishes* 89:143–150.
- Rakauskas, V., E. Bacevičius, Ž. Pūtys, L. Ložys, and K. Arbačiauskas. 2008. Expansion, Feeding and Parasites of the Round Goby, *Neogobius melanostomus* (Pallas, 1811), a recent invader in the Curonian Lagoon, Lithuania. *Acta Zoologica Lituanica* 18:180–190.
- Reusch et al. 2010. Microsatellites reveal origin and genetic diversity of Eurasian invasions by one of the world's most notorious marine invader, *Mnemiopsis leidy* (Ctenophora). *Molecular Ecology*. 19:2690-2699.
- Reuschel, S., J. A. Cuesta, and C. D. Schubart. 2010. Marine biogeographic boundaries and human introduction along the European coast revealed by phylogeography of the prawn *Palaemon elegans*. *Molecular phylogenetics and evolution* 55:765–75.
- Roche, D. G., and M. E. Torchin. 2007. Established population of the North American Harris mud crab, *Rhithropanopeus harrisi* (Gould, 1841) (Crustacea: Brachyura: Xanthidae) in the Panama Canal. *Aquatic Invasions* 2:155–161.
- Rumohr, H., Bonsdorff, E. and T.H. Pearson. 1996. Zoobenthic succession in Baltic sedimentary habitats. *Arch. Fish. Mar. Res.*, 44:179–213.
- Schubert, K. 1936. *Pilumnopus tridentatus* Maitland, eine neue Rundkrabbe in Deutschland. *Zoologischer Anzeiger* 116:320–323.
- Skora, K. E., and J. Rzeznik. 2001. Observations on Diet Composition of *Neogobius melanostomus* Pallas 1811 (Gobiidae, Pisces) in the Gulf of Gdansk (Baltic Sea). *Journal of Great Lakes Research* 27:290–299.
- Skora, K. E., and J. Stolarski. 1993. New fish species in the Gulf of Gdansk *Neogobius* sp [cf. *Neogobius melanostomus* (Pallas 1811)]. *Notes Bulletin of the Sea Fisheries Institute* 1:83.
- Tiselius, P. and L. F. Møller. 2017. Community cascades in a marine pelagic food web controlled by the non-visual apex predator *Mnemiopsis leidy*. *Journal of Plankton Research*. 39:271-279.
- Ustups, D., U. Bergström, A. B. Florin, E. Kruze, D. Zilniece, D. Elferts, E. Knospina, and D. Uzars. 2016. Diet overlap between juvenile flatfish and the invasive round goby in the central Baltic Sea. *Journal of Sea Research* 107:121–129.
- Walsh, M. G., Dittman, D. E. & O’Gorman, R. 2007: Occurrence and food habits of the round goby in the profundal zone of southwestern Lake Ontario. *Journal of Great Lakes Research* 33, 83–92
- Zettler, M. L. 2003. Benthologischer Arbeiten zur ökologischen Bewertung von Windenergie-Anlagen-Eignungsgebieten in der Ostsee. Endbericht für die Areale Kriegers Flak (KF) und Westlicher Adlergrund (WAG). FKZ: 802-85-210.

Zettler, M. L., D. Daunys, J. Kotta, and A. Bick. 2002. History and Success of an Invasion into the Baltic Sea: The Polychaete *Marenzelleria* cf. *viridis*, Development and Strategies. Pages 66–75 Invasive Aquatic Species of Europe. Distribution, Impacts and Management.

Zmudzinski, L. 1996. The effect of the introduction of the American species *Marenzelleria viridis* (Polychaeta: Spionidae) on the benthic ecosystem of Vistula lagoon. Mar. Ecol.-Pubblicazioni Della Stazione Zoologica Di Napoli I 17:221–226.

The Round Goby (*Neogobius melanostomus*)

Infection rates and prevalence of metazoan parasites of the non-native round goby (*Neogobius melanostomus*) in the Baltic Sea

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Abstract Studies in the Baltic Sea have identified over 30 parasite taxa infecting the invasive round goby (*Neogobius melanostomus* (Pallas, 1814)). In this study, we aimed at comparing parasite assemblages and infection rates (prevalence and intensity) in different populations across the invasive range in the Baltic Sea (Denmark, Lithuania, Estonia and Finland). Infection rates were 56–60% across all locations except Lithuania (28%). However, the parasite assemblages in the sampled populations were dissimilar, each location having unique parasites. In addition,

many of the parasites were generalists commonly infecting native fish species. Based on the results of this study and those previously conducted in the Baltic Sea, the round goby has not retained parasites from its area of origin, but instead has been successively colonized by native generalist parasites. Although variable, overall parasite richness is still quite low around the Baltic compared to the native areas (34 vs 71 taxa, respectively). Also, prevalence and mean infection intensities in the Baltic Sea are significantly lower than in the native areas. Therefore, the invasion success of the round goby in the Baltic Sea can at least partly be attributed to enemy release, in this case shedding a significant proportion of their native parasite load.

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Keywords Parasite prevalence · Parasite assemblages · Enemy release · Invasion · Invasion ecology

Introduction

The round goby (*Neogobius melanostomus* (Pallas, 1814)) is one of the most widespread non-native fish species in the Baltic Sea (Kotta et al., 2016), with reported detrimental biological impacts in many locations (Ojaveer & Kotta, 2015; Ojaveer et al., 2015). Following its initial detection in the Gulf of Gdansk in 1990 (Skora & Stolarski, 1993), it spread throughout the southern Baltic Sea, eastward to the

Vistula lagoon and river system (Mierzejewska et al., 2011), westward to the German and Danish coastal areas and further north along the southeastern coast of Sweden (Florin & Karlsson, 2011; Sapota, 2011). In the northern parts of the Baltic Sea, round goby became a permanent component of the Lithuanian, Latvian and Estonian coastal fish fauna in the 2000s (Ojaveer, 2006; Sapota, 2011; Rakauskas et al., 2013; Strake et al., 2013). By 2011, the species had been recorded along the Finnish coast of the Gulf of Finland, in the Archipelago Sea and the Åland Islands. The northernmost observations to date were made in 2013 outside the town of Raahe (about 64°4'N) in the Bothnian Bay. Currently, the species has been observed along the coasts of all Baltic sub-basins, except for the Swedish coast of the Bothnian Bay (Kotta et al., 2016). Although originating from the brackish waters of the Black Sea and Sea of Azov, the species has also established viable and flourishing populations in many freshwater areas throughout European river systems (e.g. van Beek, 2006; Ondračková et al., 2010; Borcharding et al., 2011; Emde et al., 2012; Huyse et al., 2015) and the North American Great Lakes (Kornis et al., 2013).

The great invasion success of the species has partly been explained by its pronounced phenotypic plasticity in terms of life-history traits (MacInnis & Corkum, 2000; Gutowsky & Fox, 2012; Brandner et al., 2013a), feeding habits (Brandner et al., 2013b) as well as tolerance of different salinity regimes (Karsiotis et al., 2012; see review by Kornis et al., 2012). Many researchers have also suggested the lack of natural enemies as a major contributing factor (Sapota & Skora, 2005; Kvach & Skóra, 2006; Kvach & Stepien, 2008; Gendron et al., 2012). Parasites are important biological regulators and occur as an inherent part of any biological community (e.g. Williams et al., 1992). They can also be seen as a good indicator of the ecosystem health (Hudson et al., 2006; Palm, 2011). The lack of such natural regulators has—in accordance with the enemy release hypothesis—been pointed out as one of the reasons to the successful colonization and population expansion of many introduced species (Torchin et al., 2003; Colautti et al., 2004). Introduced species are known to have an impact on the native fauna in not only many direct ways, e.g. through competition and predation, but also indirectly by acting as a vector for new parasites and pathogens (Ruiz et al., 1999; Emde et al., 2012). This spillover

effect of non-indigenous parasites being introduced to new ecosystems together with their non-indigenous hosts has been discussed in numerous studies (e.g. Johnsen & Jensen, 1991; Prenter et al., 2004; Peeler et al., 2010; Lymbery et al., 2014). Kelly et al. (2009) highlighted the significance of the spillback of native parasites, i.e. when non-native species act as a new competent intermediate and/or paratenic host for native parasites. This spillback may be of pronounced ecological significance, as it facilitates the transmission of parasites to native hosts and can increase the prevalence of diseases in a population (Kelly et al., 2009 and references therein). However, non-native species may also alleviate the parasitic load of native species by ‘diluting’ the intensity of infection, e.g. when replacing native host species and acting as a “resistant target” (Kopp & Jokela, 2007). Thus, non-native species can simultaneously play many different roles in the parasite–host cycle.

The round goby was most probably transported to the Gulf of Gdansk in ballast water as eggs or larvae (Sapota & Skora, 2005). It is not known whether the source population is from the Black, Azov or Caspian Sea and whether it arrived via the inland river systems of Don/Volga or Dnieper–Vistula, but the species was most likely transported to the Baltic with the ballast water of vessels (Sapota, 2004). This comparatively rapid translocation, together with a steep decline in salinity (16–18 PSU in the Black Sea vs. 7–8 PSU in the Gulf of Gdansk) may have caused the loss of most of the native parasite fauna (Kvach et al., 2014). In a recent study, Kvach et al. (2014) found that the round goby had relatively many (15 species) metazoan parasite species in its native area (the Danube estuary) compared to other gobiid species, but had retained very few when the round goby invaded Vistula delta (5 species). Conversely, the racer goby (*Babka gymnotrachelus*, Kessler, 1857) has lower parasite richness in the native than in the invasive range (Kvach et al., 2014). Many previous results, in addition to the above, show that the round goby may be benefitting from decreased parasite load, supporting the enemy release hypothesis (ERH) (Kvach, 2001; Kvach & Skóra, 2006; Francová et al., 2011; Emde et al., 2012).

Studies have shown that so far the round goby has relatively low infection rates in the Baltic (some 7–20 taxa; Rolbiecki, 2006; Rakauskas et al., 2008) compared to its native range (Kvach 2005) (up to 71 taxa and overall infection rate of up to 97%; Rolbiecki,

2006; Özer, 2007). All parasites found in the Baltic populations have also been previously recorded in the Baltic Sea (Rolbiecki, 2006; Kvach & Winkler, 2011). Therefore, there is no indication of spillover of non-native parasites into the Baltic Sea, which was an obvious concern knowing that some non-indigenous fish have introduced invasive and severely harmful parasites to native species (e.g. Johnsen & Jensen, 1991; Lymbery et al., 2014). However, in the Vistula Lagoon, the round goby has been reported to host larvae of the introduced swim bladder nematode *Anguillicoloides crassus* (Kuwahara, Niimi & Itagaki, 1974) (Kvach, 2004a; Rolbiecki, 2006), which may lead to further spread and transmission to its definitive host, the European eel (*Anguilla anguilla*, L., 1758), whose populations are already declining in the Baltic Sea (Kirk, 2003; Kvach, 2004a; Rolbiecki, 2006). While not currently found parasitizing the round goby in the Baltic Sea, the goby-specific Ponto-Caspian monogenean *Gyrodactylus proterorhini* (Ergens, 1967), has been transferred to the Vistula basin by other Ponto-Caspian gobies (Mierzejewska et al., 2011) and is more abundant in the non-native than in the native areas (Kvach et al., 2014). In other invaded fresh water habitats, like the Rhine River, round goby is also suspected to aid in the spreading of the non-native acanthocephalan parasite *Pomphorhynchus tereticollis* (Rudolphi, 1809) (Emde et al., 2012), and although this particular species is already a part of the parasite fauna in the Baltic Sea (Špakulová et al., 2011), it shows that the round goby has a potential of transmitting parasites to new areas as it continues to spread.

Populations of the round goby across the Baltic Sea are very different. They have a very different invasion history primarily in terms of time since introduction: the most recent introductions and oldest ones are more than 20 years apart (Sapota, 2011; Kotta et al., 2016). Also, the populations are exposed to very different abiotic conditions due to the pronounced gradients in both salinity and temperatures in the Baltic Sea and, therefore, different local parasite assemblages. The population age, i.e. time since introduction, is often reflected in the parasite loads as the infection rates increase the older the population. This has been true, for example, in the Gulf of Gdansk, where the parasite abundance of the round goby increased from six species in the first studies (Rokicki & Rolbiecki, 2002) to at least 12 metazoan parasite species by 2006

(Kvach & Skóra, 2006). In the most recently established populations, the infection rates are presumably still low due to fewer parasitic species adapted to this new host.

Studies focusing on round goby parasites in the Baltic Sea have been quite few, spatially limited to local studies on German, Polish and Lithuanian populations and completely lacking in the northern Baltic Sea (Kvach, 2001, 2004a; Kvach & Skóra, 2006; Rakauskas et al., 2008; Kvach & Winkler, 2011). The aim of the present study was to identify, quantify and compare the most common metazoan parasites (concentrating on metazoan endoparasites) infecting the round goby in four distinct locations in the Baltic Sea. In addition, we also review the existing literature on the round goby parasites in the region, and compare and contrast them to the results of this study.

Material and methods

Round gobies were collected from four locations around the Baltic Sea (Denmark, Lithuania, Estonia and Finland [the Åland islands]) (Fig. 1). The sampling was conducted in June 2015 using identical trap assays at each site (see below). The sites were chosen based on the previous observations of the round goby as these are the locations where the species was first discovered and populations are established and round gobies are abundant (Sapota, 2004; Ojaveer, 2006; Azour et al., 2015; Kotta et al., 2016). The habitat at all sampling locations consisted of vegetated sandy bottoms, except in Finland and Estonia where the bottom substrate consisted mainly of coarse gravel and rocks interspersed with sandy patches. This reflects a general change in dominant habitats from southern to northern Baltic Sea. The locations in Lithuania, Estonia and Finland were also close to artificial structures such as piers or built embankments, which has been noted as the round gobies preferred habitat as they provide suitable nesting sites and shelter (MacInnis & Corkum, 2000; Sapota & Skora, 2005; Ojaveer, 2006). The populations differ as to the time of invasion: the Lithuanian population around Klaipeda was discovered in 2002 (Sapota, 2011) and the population in Muuga, Estonia (Gulf of Finland) was discovered two years later in 2004 (Ojaveer, 2006). The first records of the round goby from Guldborgsund

Fig. 1 Sampling sites around the Baltic Sea (see Table 1 for more information) and the prevalence of parasite infection at each location

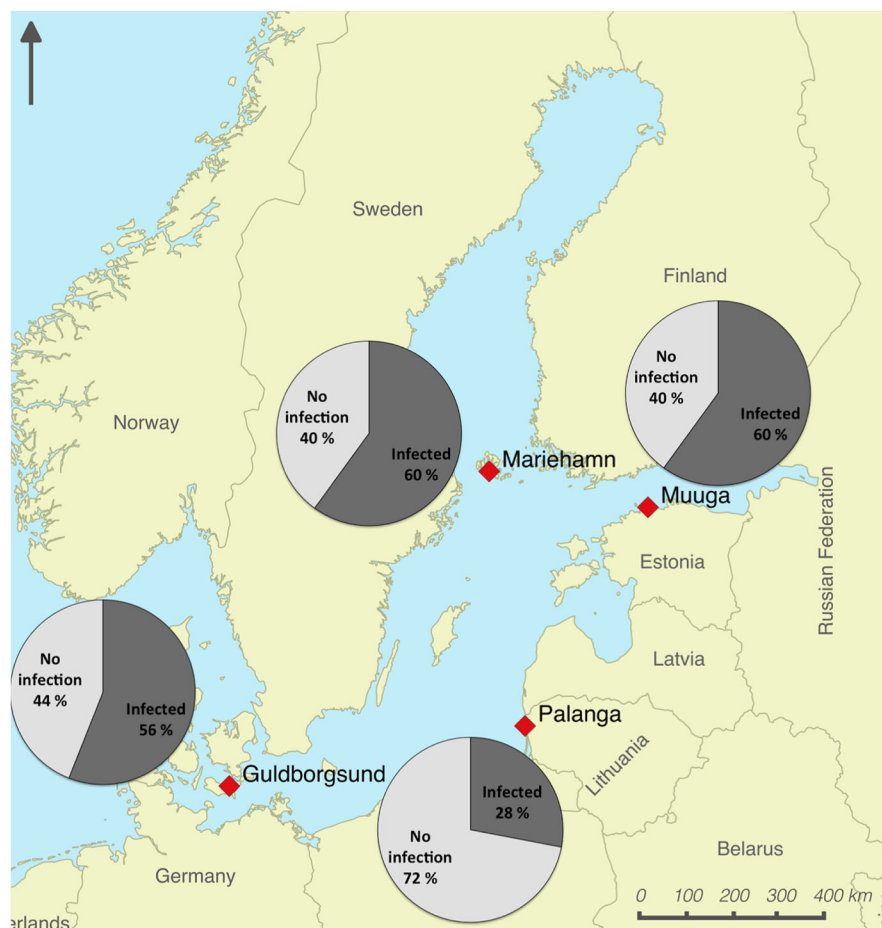


Table 1 Hydrographic data during sample collection at each site

Site	Sampling date	Temperature (°C)	Salinity (PSU)
1. Guldborgsund (DK)	25.05.2015	16.7	11.9
2. Palanga (LT)	08.06.2015	15.6	5.8
3. Muuga (EE)	16.06. and 09–17.07.2015	15.0–17.0	6.0
4. Mariehamn (FI)	26.06.2015	15.1	5.8

(GBS), Denmark, are from 2008 (Azour et al., 2015) and the population in Mariehamn, Åland islands, was first recorded in 2011 (Kotta et al., 2016).

Hydrographic information from each site at the time of sample collection is presented in Table 1. Fish were collected using three types of passive gear; 5 minnow traps (mesh size 6 mm), 10 collapsible crayfish traps (mesh size 12 mm) and 3 eel traps (mesh size in cod ends 10, 14 and 17 mm). This combination was used to provide a better size range of

catch than any trap type used alone. The traps were placed at approximately 2-m intervals parallel to the shoreline at 1–2 m depth and checked every 24 h during 3 days. A piece of frozen herring (or other locally available fish) was placed in sealed mesh bags inside the minnow and crayfish traps and baits were replaced as needed. The use of both baited and unbaited methods also gives a more representative sample of the population. The sampling procedure remained the same throughout the locations to enable

comparisons between locations. Fish from each sampling time ($n = 3$) and trap type ($n = 3$) were placed in separate bags and 3–4 fish were haphazardly taken from each bag for inspection so that a total number of 25 of round gobies per site, representing various sizes, were randomly selected for closer inspection. The collected fish were immediately terminated and frozen (-18 to -20°C) until examined. Before dissection, they were measured (total length, TL, in mm), weighed (W, in g) and their sex was determined. Their livers were extracted and weighed to obtain an estimate of health and energy reserves expressed as hepatosomatic index (HSI) ($\text{HSI} (\%) = 100 \times (\text{liver weight [g]} / \text{whole fish weight [g]})$). In addition, the condition of the inspected fish was expressed using the Fulton's K index (Fulton, 1904) as $K = 100 \times W [\text{g}] / \text{TL} [\text{cm}]^3$. Differences in fish condition between sites were tested using analysis of variances (ANOVA) and post hoc comparisons using the Tukey HSD procedure. Hepatosomatic index was log-transformed to fulfil the assumptions for parametric analyses.

The skins, fins and gills were carefully examined visually in case of signs of ectoparasites (or larval stages of endoparasites like metacercariae of digenean trematodes) and all anomalies in appearance were noted. All ectoparasite taxa are typed in italics (Tables 4 and 6) to allow for comparisons with the previously published studies. Opaque eyes can be a sign of *Diplostomum* spp. metacercariae larvae, which infect the lens of the eye and have been found in previous studies as one of the most abundant parasites of the round goby in the Gulf of Gdansk (Kvach & Skóra, 2006; Rolbiecki, 2006). The eyes of each fish were dissected and inspected carefully under a dissection microscope.

The entire intestinal tract was then removed for inspection of endoparasites on both inner and outer intestinal surfaces. The gut contents were removed and the digestive tract inspected carefully for parasites. Food items in the stomach and gut were also recorded by examining the contents under a microscope and identifying which taxa were present. The number of taxa in each stomach and the frequency of occurrence ($\text{FO} \% = \text{stomachs including prey item "i"} / \text{total number "n"} \text{ of stomachs with contents at location "x"}; \text{Hyslop, 1980}$) were calculated. This was done to get a picture of which taxa are being consumed at each location at the time of sampling. The body cavity and organs (gonads, kidney, liver, mesenteries and spleen)

were inspected under a dissection microscope. All parasites were counted and stored in 70% alcohol prior to a more detailed examination and identification. Digenean trematode larvae and Hirudinea were identified according to descriptions in Valtonen (2012). Often the Diplostomid larvae found in the lens of fish have been identified as *Diplostomum spathaceum* (Höglund & Thulin, 1982), but as studies have shown there are at least two species infecting the lens (Valtonen & Gibson, 1997), and *D. spathaceum* is most likely a conglomeration of species. Thus, all Diplostomid larvae in this study were assigned to *Diplostomum* spp. since no genetic verification was made. Nematodes and Acanthocephalans were cleared in lacto phenol before examination under a light microscope. Identification was done according to morphological descriptions of, e.g. foregut, head and tail structures of nematodes in Fagerholm (1982) and Moravec (1994). Acanthocephalans were identified according to a key (Arai, 1989) and descriptions by Valtonen (2012).

The parasitological indices were calculated according to Rózsa et al. (2000) as: prevalence (proportion of fish infected of all fish examined/site), mean intensity (MI) and median intensity (mean and median number of parasites in infected fish, MedI) and their ranges given as confidence limits obtained by bootstrapping (Rózsa et al., 2000) using the Quantitative Parasitology 3.0 software (Reiczigel & Rózsa, 2005). The differences in parasite prevalence between the sampling locations and sexes were analysed using the Fisher's exact test and the mean intensities between the locations and sexes analysed using the non-parametric Wilcoxon's test due to the skewed nature of the infection intensity data. Multiple comparisons (post hoc tests) were conducted using the non-parametric Steel–Dwass method (Critchlow & Fligner, 1991). In addition, the differences in parasite assemblages between the locations were analysed using analysis of similarities (ANOSIM) and taxa contributing the most to the observed differences were determined by similarity percentage analysis (SIMPER).

All statistical analyses were conducted using the JMP Pro 11 software (SAS Institute Inc., 2013), except the comparisons of the parasite assemblages in the infected fish between sampling locations, which were made using ANOSIM (analysis of similarities) and SIMPER (similarity percentages analysis) in Primer v6 software (Clarke & Gorley, 2006).

Results

Overall condition of the round gobies in different locations

Out of a total of 100 fish that were examined, 32 were females and 68 males (Table 2). Both males and females were the smallest in Guldborgsund (TL 64–133 mm), whereas males were the largest in Mariehamn (mean TL 152,5 ± 23,5 mm) and females in Palanga (TL 77–198 mm) (Table 2). No females

were caught in Mariehamn. The hepatosomatic Index (HSI) varied between the locations ($F(3,107) = 9.49$, $P = 0.0001$). Fish from both Muuga ($P = 0.0002$) and Mariehamn ($P = <0.0001$) had a highly significantly higher HSI than in Guldborgsund and significantly higher in Palanga ($P = 0.035$). The condition index (Fulton's K) was similar throughout the sampling locations ($F(3,96) = 0.587$, $P = 0.625$) and the infection intensity had no impact on either HSI or Fulton's K ($F(1,50) = 2.42$, $P = 0.126$ and $F(1,50) = 2.67$, $P = 0.109$, respectively).

Table 2 Number of fish, the total length (TL) and weight of round gobies inspected per site

Site	GBS		Palanga		Muuga		Mariehamn	
	Female	Male	Female	Male	Female	Male	Female	Male
N	12	13	9	16	11	14	0	25
TL (mm)	$x \pm SD$	Min–max	$x \pm SD$	Min–max	$x \pm SD$	Min–max	$x \pm SD$	Min–max
Female	96.9 ± 15.4	76–133	124.2 ± 26.9	98–180	147.2 ± 27.4	90–168	–	–
Male	95.6 ± 17.6	64–126	144.4 ± 41.6	77–198	128.1 ± 32.9	72–170	152.5 ± 27.5	93–205
Total	96.2 ± 16.3	64–133	137.2 ± 37.7	77–198	136.5 ± 31.5	72–170	152.5 ± 27.5	94–205
Weight (g)	$x \pm SD$	Min–max	$x \pm SD$	Min–max	$x \pm SD$	Min–max	$x \pm SD$	Min–max
Female	13.8 ± 7.8	5.5–34.0	30.1 ± 23.3	12–80.1	55.1 ± 23.7	8.3–76.4	–	–
Male	14.9 ± 9.8	3.1–37.9	55.2 ± 43.2	6.1–129.1	34.9 ± 24.4	4.8–70.5	55.2 ± 25.9	18.7–121.4
Total	14.4 ± 8.7	3.1–37.9	46.1 ± 38.7	6.1–129.1	43.8 ± 25.7	4.8–76.4	55.2 ± 25.1	18.7–121.5

Length and weight are presented as mean and standard deviation together with the range for each parameter. Each parameter is given separately for both sexes and as a total per location

Table 3 Number of infected and non-infected fish, prevalence (%) of infection, and mean and median intensities of infections (MI and MedI, respectively) at each location

Site	Sex	N	Infected	Prev.	Lower CL	Upper CL	MI	Lower CL	Upper CL	MedI	Lower CL	Upper CL
Guldborgsund		25	14	0.56	0.35	0.76	9.14	4.71	15.30	5	2	5
	M	13	6	0.46	0.19	0.75	7.50	2.17	21.80	3.5	1	31
	F	12	8	0.67	0.35	0.90	10.40	4.38	19.90	5	2	26
Palanga		25	7	0.28	0.15	0.54	2.12	1.00	4.75	1	1	3
	M	16	4	0.25	0.07	0.52	1.50	1.00	2.00	1	–	–
Muuga		25	15	0.60	0.39	0.79	6.60	3.20	7.67	3	2	9
	M	14	7	0.50	0.23	0.77	5.29	1.86	10.10	2	1	16
	F	11	8	0.73	0.39	0.94	5.00	2.88	7.62	4	1	9
Mariehamn		25	15	0.60	0.39	0.79	7.93	4.00	16.50	3	1	7
	M	25	15	0.60	0.39	0.79	7.93	4.00	16.50	3	1	7
	F	0	–	–	–	–	–	–	–	–	–	–

The range is expressed as confidence limits (CL)

Parasite prevalence, infection intensity and assemblages in different locations

In total 51% of the fish examined were infected by at least one parasite taxa (Table 3). Overall, 383 specimens of metazoan parasites representing 10 taxa (3 species of Trematoda (Digenea), 3 Nematoda, 3 Acanthocephala and 1 Hirudinea) were identified (Table 4).

In Muuga, 73% of all females were infected, whereas the total prevalence was 60% in both Muuga and Mariehamn (Fig. 1). These two sites had the highest prevalence, compared to 56% in GBS and 28% in Palanga (Fig. 1, Table 3), although the differences were non-significant (Fisher's exact test,

2-sided, $P = 0.164$). Similarly, there was no significant difference in prevalence between the sexes (Fisher's exact test, 2-sided, $P = 0.199$). The observed numbers of parasite taxa were similar throughout the locations: three in GBS and Muuga, four in Palanga and five in Mariehamn (Table 4). The populations in Muuga and Mariehamn had the highest infection intensity (number of parasites in one fish) with a maximum of 99 and 121 parasite individuals observed. Overall infection intensity was very close to significantly higher in females than males ($Z = 1.93$, $P = 0.052$). The infection intensity (mean intensity) varied between the locations ($X^2(3, N = 52) = 8.29$, $P = 0.040$; Table 5). These differences are mainly driven by Palanga, which had significantly lower

Table 4 Parasites of *N. melanostomus* at four locations in the Baltic Sea

Parasite species, stage	Location	Guldborgsund			Palanga			Muuga			Mariehamn		
		P	MI	I	P	MI	I	P	MI	I	P	MI	I
Trematoda (Digenea)													
<i>Cryptocotyle</i> sp., met. ^{ba} (Creplin, 1825)	Skin, fins	40	9	5–30									
<i>Diplostomum</i> spp., met. (Rudolphi, 1819)	Eye, lens							52	6	1–16	40	10	1–36
<i>Tylodelphys clavata</i> , met. ^b (Nordmann, 1831)	Vitreous body							12	4	1–7			
Nematoda													
<i>Camallanus</i> sp., ad.	Intestine										8	2	1–2
<i>Contraecaecum</i> spp., L3	On intestine	12	2	1–4									
<i>Hysterothylacium aduncum</i> L3, L4, ad. (Rudolphi, 1802)	Intestine, mesentery				16	3	1–7						
Ascaridoidea indet., encysted larvae	Intestinal wall, mesenteries, body cavity	16	1	1	4	1	1	12	1	1	12	1	1
Acanthocephala													
<i>Corynosoma</i> sp., cysth.	Intestine										4	1	1
<i>Echinorhynchus gadi</i> , ad. (Müller, 1776)	Intestine				4	1	1						
<i>Pomphorhynchus laevis</i> , cysth. (Müller, 1776)	Intestinal mesentery				8	1	1						
Hirudinea													
<i>Piscicola geometra</i> ^c (L., 1761)	Skin										4	1	1–2
	Total	56	9	1–31	28	2	1–8	60	7	1–16	60	8	1–36

The primary site of infection, prevalence in percent (P), mean intensity (MI) and intensity (min–max) is given for each species/taxa, as well as, a total at each site

Met metacercariae larvae; *L3*, *L4* stage 3 and 4 larvae; *cysth* cystacanth larvae; *ad* adult

^a Metacercariae capsules (black pigmentation) counted

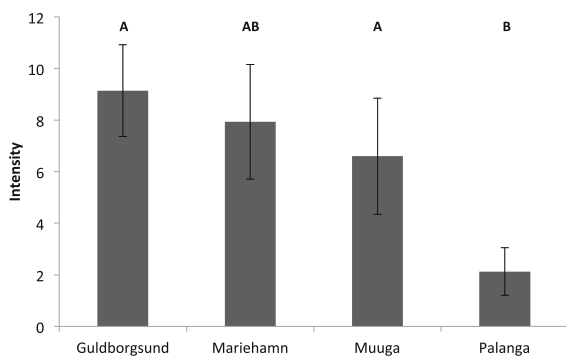
^b Identification based on ecological characteristics

^c Found unattached to fish, in the bag with sampled fish

Table 5 Results of non-parametric analyses of variances (Wilcoxon's test) and multiple comparisons (Steel–Dwass method) of the mean intensities of parasite infection between the different locations

Wilcoxon test	X^2	DF	<i>P</i> value	<i>P</i> value
Overall	8.29	3	0.0404	
Multiple comparisons	Mean difference	SE dif	Z	
Muuga: Mariehamn	2.6	3.19	0.82	0.8474
Muuga: Guldborgsund	-1.38	3.13	-0.44	0.9713
Mariehamn: Guldborgsund	-2.21	3.12	-0.71	0.8938
Palanga: Mariehamn	-4.89	2.80	-1.75	0.2999
Palanga: Guldborgsund	-7.17	2.78	-2.58	0.0485
Palanga: Muuga	-7.95	2.92	-2.73	0.0324

Significant differences are indicated in bold

**Fig. 2** Differences in mean intensity (MI) of parasite infection between the sampling locations. Variability expressed as standard error. Same letters shared among the groups indicate no significant difference according to the Steel–Dwass method (post hoc test)

mean intensities than Guldborgsund and Muuga (Fig. 2; Table 5).

The non-parametric analysis of similarities shows that parasite assemblages were significantly dissimilar (Global $R = 0.442$, $P = 0.001$). Dissimilarity was largest between Muuga and Palanga ($R = 0.753$, $P = 0.001$; Table 6) and smallest (NS) between

Muuga and Mariehamn (Table 6). Analysis of Similarity Percentages (SIMPER) revealed that the differences were driven largely by the differences in abundance of *Diplostomum* spp. and their absence in Palanga, as well as, the presence of *H. aduncum* in Palanga (Table 4). Guldborgsund had the lowest similarity with all other sites, which was primarily explained by the presence of *Cryptocotyle* sp. in Guldborgsund and its absence elsewhere (Table 4). All sampled populations hosted at least one unique parasite taxa, which was not found in any of the other populations.

Parasite taxa found in the different locations

Guldborgsund, GBS (Denmark)

In Guldborgsund, 14 of the 25 fish inspected (56%) were infected by parasites (Table 3). Mean intensity was 9 and maximum intensity was 31 individuals (Table 4). The most common parasite in the round goby population, infecting 40% of the fish inspected, was the metacercariae larvae of *Cryptocotyle* sp.

Table 6 ANOSIM table of Global R values and their significance indicating differences in parasite assemblages between the sites

	Guldborgsund	Palanga	Muuga	Mariehamn
Guldborgsund		0.549***	0.747***	0.405***
Palanga	<i>0.549***</i>		0.753***	0.306***
Muuga	<i>0.747***</i>	<i>0.753***</i>		NS
Mariehamn	<i>0.405***</i>	<i>0.306***</i>	NS	

NS not significant

Italic values are indicated ($P > 0.05$)

*** $P < 0.0001$

infecting the skin and fins of the fish with an intensity of up to 30 capsules per fish (MI = 9) (Table 4). The infection causes a change in pigmentation of the host's skin around the cysts, which are seen as distinct black spots (black spot disease) on the skin. This is a typical infection of *Cryptocotyle concavum/lingua* (Creplin, 1825), which has been observed infecting the round goby, as well as, other fish in the southwestern Baltic Sea (Køie, 1999; Rokicki & Rolbiecki, 2002; Unger et al., 2014). All other parasites were larval stages of nematodes and occurred on the intestinal mesenteries or the body cavity at low intensities (1–4 individuals). Nematodes of the genus *Contracaecum* infected 12% of the round gobies in Guldborgsund. Based primarily on the size and site of infection, at least four of these were considered to be *Contracaecum rudolphii* A or *Contracaecum rudolphii* C ($P = 4\%$, $I = 4$). Four encapsulated larvae of an unidentified ascaridoidean nematode species were also found in the mesenteries and body cavity of four round gobies ($P = 16\%$) (Table 4). Most infected fish only hosted one species of parasite but three individuals were infected by both *Cryptocotyle* metacercariae and nematode larvae.

Palanga (Lithuania)

In Palanga, 7 of 25 fish inspected (28%) were infected by parasites. The species richness was four species with very low intensity, ranging between 1 and 8 individuals (MI = 2; Tables 3, 4). The most common parasite was the nematode *Hysterothylacium aduncum* ($P = 16\%$), which had a maximum intensity of 7 individuals in one host (MI = 3). Both larvae and adults were identified. One unidentified ascaridoidean larvae was found in the mesentery ($P = 4\%$). In addition to nematodes, two species of acanthocephalans were identified. Cystacanth stages of *Pomphorhynchus laevis* (Müller, 1776) were found in the intestinal mesenteries of two fish ($P = 8\%$). One adult *Echinorhynchus gadi* (Müller, 1776) was found in the intestine of a round goby ($P = 4\%$) (Table 4). One individual hosted both *H. aduncum* and *P. laevis*; all others were only infected by one species.

Muuga (Estonia)

In Muuga, 15 of 25 fish inspected (60%) were infected by parasites (MI = 7; Table 3). *Diplostomum* spp. was the most prevalent parasite species infecting 52%

of the round gobies (Table 4). Another digenean trematode species, *Tylodelphys clavata* (Nordmann, 1831), was found in 12% of the fish. The metacercariae larvae of *Diplostomum* spp. occurred in the eye lenses of the fish, whereas *T. clavata* infects the vitreous body. The mean and maximum intensities for these two parasites were 6 and 16, and 4 and 7 individuals, respectively (Table 4). In addition, a few encapsulated ascaridoidean larvae were found embedded in the intestinal wall and mesenteries of the fish ($P = 12\%$). In Muuga, 16% of the fish hosted either both species of digenean trematode larvae or digenean trematode larvae and nematode larvae simultaneously, at intensities varying between 2 and 15. The total mean intensity was 7 and the maximum observed was 16 individuals per one host (Tables 3, 4).

Mariehamn (Finland)

In Mariehamn, 15 of 25 fish inspected (60%) were infected by parasites. The most common parasite, *Diplostomum* spp. ($P = 40\%$), was the same as in Muuga (Table 4). In contrast, the parasite richness (5 species), together with the maximum intensity (36 individuals) were the highest among all sites (Table 4). Three of the parasite taxa were unique and one of these (*Corynosoma* sp.; Acanthocephala) has not been found in the round goby before. Only one immature individual of *Corynosoma* sp. ($P = 4\%$) was found in the intestine of a round goby. Another of the species not found elsewhere was the nematode *Camallanus* sp., which was found in 8% (MI = 2) of the round gobies and the third species was the hirudinean *Piscicola geometra* L., 1761. *Piscicola geometra* was also the only ectoparasite encountered. Although the two individuals found were no longer attached to the fish hosts when thawed, it is clear they had been attached at the time of capture. Three round gobies ($P = 12\%$, MI = 1; Table 4) also hosted encapsulated ascaridoidean larvae in their intestinal wall. The majority of round gobies hosted only one parasite species, but 8% were parasitized by both *D. spathaceum* and *Camallanus* sp.

Discussion

Currently, 34 metazoan parasite species have been found infecting the round goby within the invaded

Table 7 Infection rate by parasites of *N. melanostomus* around the Baltic Sea basin based on the existing literature and the present study

	Prior literature				Present study			
	Gulf of Gdansk ^{a,b,c}	Vistula lagoon/delta ^{d,g,h}	SW Baltic ^k (German coast) ^f	Curonian lagoon/Klaipeda strait ^e	Guldborgsund	Palanga	Muuga	Mariehamn
Monogenea								
<i>Gyrodactylus rugiensis</i> , ad.			X					
Cestoda								
<i>Bothriocephalus</i> sp.(<i>scorpii</i>), pl.	X	X	X					
<i>Eubothrium crassum</i> , pl.		X						
<i>Paradilepis scolecina</i> , pl.		X						
<i>Proteocephalus filicollis</i>		X						
<i>Proteocephalus gobiorum</i>		X						
<i>Proteocephalus</i> sp.		X		X				
Trematoda (Digenea)								
<i>Bucephalus polymorphus</i> , met.			X					
<i>Bunodera luciopercae</i>		X						
<i>Cryptocotyle</i> spp, met.	X	X			X ⁱ			
<i>Diplostomum</i> spp., met.	X	X	X	X			X	X
<i>Tylodelphys clavata</i> met.	X	X					X	
<i>Tylodelphys</i> sp., met.			X					
Nematoda								
<i>Anguillicoloides crassus</i> , L3	X	X						
<i>Camallanus truncatus</i>		X						
<i>Camallanus lacustris</i>				X				X
<i>Contraecum</i> spp., L3		X			X			
<i>Contraecum rudolphii</i> L3		X						
<i>Cosmocephalus obvelatus</i> L3		X	X					
<i>Cystidicoloides ephemeridarum</i>		X						
<i>Dichelyne minutus</i>	X	X						

Table 7 continued

	Prior literature				Present study			
	Gulf of Gdansk ^{a,b,c}	Vistula lagoon/delta ^{d,g,h}	SW Baltic ^k (German coast) ^f	Curonian lagoon/Klaipeda strait ^e	Guldborgsund	Palanga	Muuga	Mariehamn
<i>Eustrongylides excisus</i> L3			X					
<i>Hysterothylacium aduncum</i>, L3, L4, ad.	X	X		X		X		
<i>Paracuaria adunca</i> , L3			X					
<i>Agamonema</i> sp. L3		X						
Ascaridoidea indet.					X	X	X	X
Acanthocephala								
<i>Acanthocephalus anguillae</i>				X				
<i>Acanthocephalus lucii</i>	X							
<i>Echinorhynchus gadi</i>	X	X	X	X		X		
<i>Corynosoma</i> sp.								X
<i>Pomphorhynchus laevis</i>	X	X	X	X		X		
Hirudinea								
<i>Piscicola geometra</i>	X							X
Crustacea								
<i>Ergasilus sieboldi</i>	X							
Bivalvia								
<i>Unio</i> sp., glochidia		X	X					
Total	13	22	11	7	3	4	3	5
Infection rate	98.7% ³	18.3 ^d	58.10% ^{if}	–	56%	28%	60%	60%

The most common parasites are in bold and ectoparasites in grey

Met metacercariae larvae; L3, L4 stage 3 and 4 larvae; *cysth* cystacanth larvae; *pl* plerocercoid larvae; *ad* adult

^a Rokicki & Rolbiecki, 2002, ^bKvach, 2001, ^cKvach & Skóra, 2006, ^dRolbiecki, 2006 (and references therein), ^eRakauskas et al., 2008, ^fKvach & Winkler 2011, ^gKvach et al., 2014, ^hSzostakowska & Fagerholm, 2007

ⁱ Identification based on ecological characteristics

^j Value given in Kvach & Winkler, (2011) including microsporidians

^k Area of study includes: Szczecin Lagoon, Peenemünde (Peene river), Strelasund strait, Unterwarnow (Mecklenburg bight) and Kiel Canal

Baltic Sea basins and lagoons (Kvach, 2001; Rokicki & Rolbiecki, 2002; Kvach & Skóra, 2006; Rolbiecki, 2006; Rakauskas et al., 2008; Kvach & Winkler, 2011; Table 7). Most of the taxa have been detected in the Gulf of Gdansk, and Vistula lagoon and river delta (13

and 22 taxa respectively; Table 7). This is expected, as these areas have been inhabited by round gobies the longest, since the early 1990s. Previously, the lowest species richness was reported for the Curonian lagoon, where only 7 parasitic species were found (Rakauskas

et al., 2008). In the Gulf of Gdansk, round goby populations have shown a clear increase with time in the number of parasites and intensity of infection. The first study in the region only showed five taxa of metazoan endoparasites infecting the round goby (Rokicki & Rolbiecki, 2002), whereas, a few years later, 12 taxa (8 endoparasite species and 4 ectoparasites) were recorded (Kvach & Skóra, 2006). The later study also had an infection rate of 98.7% (Kvach & Skóra, 2006), which closely resembles the infection rate of 97.5% reported by Özer (2007) in the goby's native range in the southern Black Sea. However, the mean intensity of parasite infections was much higher (78.2 ± 23.1) in the Black Sea than in the Gulf of Gdansk. In the invasive range, the round goby also tends to have a lower prevalence of parasites compared to other gobiid species and other fish species (Kvach, 2001, 2004b; Ondračková et al., 2010; Gendron et al., 2012). In the southwestern Baltic region, both species richness (11 species; Table 7; Kvach & Winkler, 2011) and infection rates (57%; Table 7; Kvach & Winkler, 2011) are lower than in other gobiid species, which all host a range of 14–24 parasite species and have infection rates regularly attaining 100% (Zander, 2003).

The parasite taxa richness in the present study is quite low (3–5 species; Tables 4, 7). Nevertheless, many of the most common species found in the present study are the same as in the previous investigations. Digenean trematode larvae of the species *Diplostomum* spp. have been observed as the most prevalent parasites in many previous studies in the Baltic Sea (Kvach & Skóra, 2006; Kvach & Winkler, 2011; Kvach et al., 2014; Table 7), as well as, other native and non-native areas (Kvach & Skóra, 2006; Kvach & Stepień, 2008; Francová et al., 2011; Gendron et al., 2012). In this study, it was found to be the most common parasite with the highest prevalence and intensity in Muuga and Mariehamn (Table 3), but absent from the two other sites. *Diplostomum* spp. together with another digenean trematode species, *Cryptocotyle* sp. observed in Guldborgsund, were the only species reaching high prevalence and intensities (Table 4).

Of the nematode species observed in this study, *Hysterothylacium aduncum* has frequently been reported infecting the round goby in all areas except the southwestern Baltic Sea (Kvach & Winkler, 2011; Table 7). This is despite the fact that *H. aduncum* does

occur quite commonly in the area and has been recorded in native goby species (Zander, 2003). The acanthocephalans *Echinorhynchus gadi* and *Pomphorhynchus laevis*, which were found in Palanga (Table 4), are also common species reported for the round goby in the Baltic Sea, as well as, other brackish and freshwater native and non-native areas (Kvach & Skóra, 2006; Francová et al., 2011; Table 7). *Hysterothylacium aduncum* and *E. gadi* are both fish parasites of marine origin which are commonly found in eelpout (*Zoarces viviparus* L., 1758) and cod (*Gadus morhua* L., 1758), but can also be found in other fish species (Fagerholm, 1982; Valtonen, 2012).

Larvae of *Contracaecum osculatum*, a mammalian parasite infecting mostly seals, have been observed in the round goby in the Vistula lagoon (Rolbiecki, 2006), but the distinction between *C. osculatum* and *C. rudolphii* larvae is often difficult and thus some specimens may have been misidentified. However, *C. rudolphii* found in this study in Danish and Lithuanian round gobies is an avian parasite, maturing primarily in cormorants (Szostakowska & Fagerholm, 2007, 2012). Cormorants have been known to prey actively on round gobies where they co-occur, e.g. in the Gulf of Gdansk (Bzoma, 1998) and the Curonian Lagoon (Rakauskas et al., 2013). Thus, round goby is a potential paratenic host in completing the life cycle of this *C. rudolphii* in the Danish and Lithuanian populations (Table 4).

This study gives a new host record for the acanthocephalan *Corynosoma* sp. parasitizing round gobies in the Baltic Sea. The taxon was observed in Mariehamn, Finland (Table 4). The three species in the genus *Corynosoma* occurring in the Baltic Sea are all primarily seal parasites and mainly use the amphipod *Monoporeia affinis* (Lindström, 1855) and sculpins or other fish as the intermediate and paratenic hosts (Valtonen, 2012). So far, there has been no evidence of seals using round gobies as prey. However, the migration patterns of the round goby in the Baltic Sea are still poorly understood and it is possible that during autumn and early winter as they migrate to deeper water and possibly further offshore, seals could prey on round gobies. Thus, the round goby may act as an alternative host in the life cycle of *Corynosoma* sp. It is also possible that the round goby is merely a “dead-end” paratenic host for these species and could thus contribute to a diluting effect for these parasites if they do not reach their definitive hosts. However, with

such low infection intensities in the round goby, the aforementioned ecological effects are likely to be restricted.

The observed parasite species are all generalists and occur commonly in the Baltic Sea. The fact that the round goby shows lower infection rates than other benthic fish species in the invaded area (Kvach & Winkler, 2011), together with the lack of any specialized and/or non-native parasites (Kvach, 2001; Kvach & Skóra, 2006), complies with the theory of enemy release in the Baltic populations (Torchin et al., 2003; Colautti et al., 2004). We suggest that a similar increase in parasite infection prevalence and intensity, as was seen in the Gulf of Gdansk, may be expected in the northern populations, sampled in this study, as the native parasites gradually adapt to and colonize this new host species.

The Baltic Sea parasite fauna, similarly to the Baltic Sea fauna and flora in general, is a heterogeneous assemblage of species of freshwater, marine and brackish water origin (Fagerholm, 1982; Kjøie, 1999; Zander & Reimer, 2002). Some changes in the parasitic fauna of the round goby can thus be observed due to differing salinity in the southern/southwestern population (Denmark) compared to the northern populations (Estonia and Finland). The increase of *Diplostomum* spp. and decrease of *Cryptocotyle* sp. has often been observed in studies following a southwest to northeast salinity gradient in the Baltic Sea (Kjøie, 1999; Unger et al., 2014). The low similarity between the locations (Tables 3, 4 and 7) might thus be explained by the difference in salinity, which causes significant differences in benthic fauna composition and, consequently, in parasite fauna that rely on these species as intermediate and paratenic hosts (Williams et al., 1992; Zander & Reimer, 2002). The parasite composition is probably a reflection of the salinity tolerance of both the parasite species and its intermediate host species. It would seem that in general the infection rate is higher in low and intermediate salinities, like in the Vistula and Szczecin lagoons (Rolbiecki, 2006; Kvach & Winkler, 2011; Table 7), as well as, in Mariehamn and Muuga (Table 3). The round goby seems to host a mix of parasites of both limnic and marine origin throughout the Baltic Sea, albeit clearly dominated by a few limnic species.

Due to complex life histories of many parasites, completing their life cycle depends on access to

suitable hosts in the system. In this study, the lack of gastropods in the diet of the round goby in Palanga (FO = 5.6%; Table 8), indicates an absence of gastropod species in the habitat and thus appeared to be reflected in an absence of some parasite species, especially digenean trematodes of the genera *Diplostomum*, *Tylodelphys* and *Cryptocotyle*, which use gastropods as intermediate hosts for the metacercariae larvae (Zander, 2003; Valtonen & Gibson, 1997). The lower infection rate in Lithuania (28%) is probably a result of this absence of digenean trematode larvae, which account for the highest prevalence at all other sites (Table 4). The absence of *Diplostomum* spp. metacercariae in Palanga is noteworthy, since it was the most prevalent parasite (13.5%) in 2007 (Rakauskas et al., 2008) and has also been reported in other fish species along the Lithuanian and Latvian coasts (Tabolina, 1994; Kjøie, 1999).

The lack of copepods in the diet of *N. melanostomus* at the study locations (Table 8) is a probable cause of the lack of cestode parasites observed in this study, since planktonic crustaceans are their primary intermediate hosts (Scholz, 1999). Together with the absence of cestode species, the lack of metazoan ectoparasites in this study is also contributing to the low species richness. The most likely reason is salinity as well as time since introduction. Most metazoan ectoparasites that have previously been found infecting the round goby in the Baltic Sea (crustacean *Ergasilus sieboldi*, Nordmann 1832 and the glochid stages of unionid bivalves) are freshwater species. The salinity in the sampling areas (5 to 7 PSU; Table 1), is thus probably too high for these species to occur. The monogenean *Gyrodactylus rugiensis*, on the other hand, is a marine parasite normally infecting *Pomatoschistus microps*, and it has previously been found infecting the round goby in salinities above 9 PSU in the Baltic (Kvach & Winkler, 2011). Monogeneans are in general quite host specific (Poulin, 2002; Huyse et al., 2003), and the introduction of the round goby in these locations happened 10–15 years ago, which is likely not enough time for most native monogeneans to adapt or switch host. The goby-specific monogenean, *Gyrodactylus proterorhini*, infects the round goby in its native areas in Black sea and Sea of Azov, but has to date not been observed in the Baltic round gobies (Mierzejewska et al., 2011). Other metazoan ectoparasites, mainly copepods, e.g. *Ergasilus sieboldi*, are found only occasionally even in

Table 8 The frequency of occurrence (%) of prey items (taxa) in the stomach contents of round gobies examined for parasites at four different locations in the Baltic Sea

Taxa	Guldborgsund			Palanga			Muuga			Mariehamn	
	Total 22	F 10	M 12	Total 18	F 7	M 11	Total 22	F 11	M 11	Total 21	M 21
<i>Mytilus</i>	4.5		8.3	22.2	14.3	27.3	13.6		27.3	9.5	9.5
<i>Macoma</i>							45.5	72.7	18.2	19.0	19.0
Bivalvia indet.	4.5		8.3								
Amphibalanus	9.1	10.0	8.3	50.0	14.3	72.7	54.5	27.3	81.8	42.9	42.9
Gastropoda	50.0	50.0	50.0	5.6		9.1	13.6	9.1	18.2	66.7	66.7
Amphipoda				61.1	28.6	81.8	18.2		36.4	9.5	9.5
Isopoda	9.1	10.0	8.3	22.2	28.6	18.2					
Decapoda	9.1	10.0	8.3	16.7		27.3				4.8	4.8
Polychaeta	22.7	30.0	16.7	11.1	28.6		9.1	9.1	9.1		
Oligochaeta	4.5	10.0		5.6	14.3		9.1	9.1	9.1	4.8	4.8
Insecta	18.2	10.0	25.0	5.6		9.1	18.2	9.1	27.3	9.5	9.5
Other	9.1	10.0	8.3								
<i>N. melanostomus</i> ^a	72.7	60.0	83.3	33.3	14.3	45.5	22.7	9.1	36.4	23.8	23.8
Pisces undetermined ^a	9.1		16.7				9.1	18.2		14.3	14.3
Pisces eggs	4.5	10.0									

At each location, the number of fish with stomach contents is given (*n*). All values are given separately for males and females as well as in total for each location

^a Scales and bones

its native area (Kvach, 2004b; Rolbiecki, 2006 and references therein; Özer, 2007). The only ectoparasite in this study, the hirudinea *P. geometra*, is very common in the Baltic Sea and has low host specificity as it infects several species of fish, e.g. eel (Rolbiecki, 2006), eelpout *Zoarces viviparus* (L., 1758), and Baltic flounder *Platichthys flesus* (L., 1758) (Køie, 1999). However, it has to be noted that the relatively small sample size (25 fish/site) can have caused some rare species not to be detected.

The most prevalent parasites in Guldborgsund, Muuga and Mariehamn, digenean trematodes, also correspond to the predominant prey items, i.e. gastropods, at these sites (FO = 50, 13.6, and 66.7%, respectively; Table 8). Likewise, the occurrence of amphipods in the stomach contents in Palanga (FO = 61.1%; Table 8) is seen in the presence of acanthocephalan parasites (Table 4). Although characterized as an opportunistic feeder (Rakauskas et al., 2008; Järv et al., 2011), the main components of the round goby diet are usually molluscs and adult barnacle *Amphibalanus improvisus* Darwin, 1854,

which can locally be a dominating prey species (Herlevi, unpubl. data; Table 8). *Contracaecum osculatatum* may infect larval stages of *A. improvisus*, but parasites which would use adult *A. improvisus* as an intermediate host are not known. There are also no other known predators for adult *A. improvisus*, although it is occasionally consumed by benthic predators as a fouling species on, e.g. *Mytilus trossulus* L., 1758 (Laudien & Wahl, 1999). A few freshwater digenean trematodes parasitizing fish and using bivalves as intermediate hosts have been found in the round goby (*Bucephalus polymorphus*, *Bunodera luciopercae*; Table 7), but these species require a freshwater bivalve species as vectors (*Dreissena polymorpha* Pallas, 1771 or *Pisidium* spp.). None of the common parasites infecting bivalves such as *Mytilus trossulus*, *Macoma balthica* L., 1758 or *Cerastoderma* spp. in the Baltic have been observed to infect round gobies (Zander & Reimer, 2002). As these species most commonly share habitat and are important prey of the round goby, it is possible that the round gobies are not exposed to many parasites

capable of infecting them. This can explain the low intensity of infection in the Baltic Sea. The diet composition of the round goby also explains some of the differences compared to native gobies, since other native goby species are parasitized mainly through ingesting intermediate hosts such as planktonic crustaceans, amphipods and oligochaetes. Thus, the reason for lower parasitization may be the round goby's main prey items and habitat (Zander, 2003; Emde et al., 2014).

The diet of the round goby and the regional differences therein thus seem to explain the intensity of parasitization and parasite assemblages observed in this study. The pattern of parasitization would indicate that the round goby has found a niche, which enables it to escape parasitization by many common parasites in the Baltic Sea. However, since the diet of the round goby has been shown to vary both seasonally and between size groups (e.g. Skora & Rzeznik, 2001; Rakauskas et al., 2008, 2013; Brandner et al., 2013b; Ustups et al., 2015), future parasitological studies should investigate both seasonal and size-related differences in the infection rates of the round goby in the Baltic Sea.

Conclusions

The round goby acts as an intermediate and paratenic host for most of the parasite species found in this study. However, the presence of adult individuals of *Hysterothylacium aduncum*, *Camallanus* sp., and *Echinorhynchus gadi* shows that it can also function as a definitive host for these parasite species. Based on this study and others previously conducted in the Baltic Sea, it seems that overall the round goby has not retained parasites from its area of origin, but instead has been successively colonized by generalist parasites in the introduced areas. Although variable, overall parasite richness is still quite low in the Baltic compared to the native areas and a similar pattern can be seen in the prevalence and mean intensities of infection (Kvach, 2001, 2004b; Özer, 2007). The present study thus adds to the evidence supporting the enemy release hypothesis (e.g. Kvach & Stepien, 2008; Emde et al., 2014; Kvach et al., 2014), as no non-native parasites were detected, and the infection rates remain quite low throughout the invaded areas. The diet and opportunistic feeding behaviour, which

enable the round goby to exploit prey items largely unutilized by other predators, are probably a key factor for the observed low parasite intensities, and continued success as an invasive species in the Baltic Sea.

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References

- Arai, H. P., 1989. Acanthocephala, p. 1–90. In L. Margolis & Z. Kabata (eds), Guide to the parasites of fishes of Canada. Part III. Canadian Special Publication of Fisheries and Aquatic Sciences 107: 95 p.
- Azour, F., M. van Deurs, J. Behrens, H. Carl, K. Hüsey, K. Greisen, R. Ebert & P. Møller, 2015. Invasion rate and population characteristics of the round goby *Neogobius melanostomus*: effects of density and invasion history. *Aquatic Biology* 24: 41–52.
- Borcherding, J., S. Staas, S. Kruger, M. Ondrackova, L. Slapansky & P. Jurajda, 2011. Non-native Gobiid species in the lower River Rhine (Germany): Recent range extensions and densities. *Journal of Applied Ichthyology* 27: 153–155.
- Brandner, J., A. F. Cerwenka, U. K. Schliewen & J. Geist, 2013a. Bigger Is Better: Characteristics of Round Gobies Forming an Invasion Front in the Danube River. *PLoS ONE* 8(9): e73036.
- Brandner, J., A. F. Cerwenka, U. K. Schliewen & J. Geist, 2013b. Comparative feeding ecology of invasive Ponto-Caspian gobies. *Hydrobiologia* 703: 113–131.
- Bzoma, S., 1998. The contribution of round goby (*Neogobius melanostomus* Pallas, 1811) to the food supply of cormorants (*Phalacrocorax carbo* Linnaeus, 1758) feeding in the Puck Bay. *Bulletin of the Sea Fisheries Institute* 2: 39–47.
- Clarke, K.R. & R.N. Gorley, 2006. User Manual/Tutorial. PRIMER-E v6, Plymouth, 192 pp. <http://www.primer-e.com/>.

- Colautti, R. I., A. Ricciardi, I. A. Grigorovich & H. J. MacIsaac, 2004. Is invasion success explained by the enemy release hypothesis? *Ecology Letters* 7: 721–733.
- Critchlow, D. E. & M. A. Fligner, 1991. On distribution-free multiple comparisons in the one-way analysis of variance. *Communications in Statistics – Theory and Methods* 20: 127–139.
- Emde, S., S. Rueckert, H. W. Palm & S. Klimpel, 2012. Invasive Ponto-Caspian Amphipods and Fish Increase the Distribution Range of the Acanthocephalan Pomphorhynchus tereticollis in the River Rhine. *PLoS ONE* 7: e53218.
- Emde, S., J. Kochmann, T. Kuhn, M. Plath & S. Klimpel, 2014. Getting What Is Served? Feeding Ecology Influencing Parasite-Host Interactions in Invasive Round Goby *Neogobius melanostomus*. *PLoS ONE* 9: e109971.
- Fagerholm, H.-P., 1982. Parasites of fish in Finland. IV. Nematodes. *Acta Academiae Aboensis, Series B* 40: 1–128.
- Florin, A.-B. & M. Karlsson, 2011. Svartmunnad smörbult i svenska kustområden. *Finfo.*, https://www.slu.se/PageFiles/98817/Finfo_2011_2_mindre.pdf.
- Francová, K., M. Ondračková, M. Poláčik & P. Jurajda, 2011. Parasite fauna of native and non-native populations of *Neogobius melanostomus* (Pallas, 1814) (Gobiidae) in the longitudinal profile of the Danube River. *Journal of Applied Ichthyology* 27: 879–886.
- Fulton, T. W., 1904. The rate of growth of fishes. *Fisheries Board of Scotland, Annual Report* 22 part 3: 141–241.
- Gendron, A. D., D. J. Marcogliese & M. Thomas, 2012. Invasive species are less parasitized than native competitors, but for how long? The case of the round goby in the Great Lakes-St. Lawrence Basin. *Biological Invasions* 14: 367–384.
- Strake, S., Perkons, V., Yermakov, V., Minde, A., Kazmers, I., Kruze, E., Romanovich, S., Medne, R., Zagars, M., Ojaveer, H., Kotta I., Jaanus, A. & A. Pöllumäe, 2013. Report on the field studies on alien species: GES-REG Project partners, 57 pp.
- Gutowsky, L. F. G. & M. G. Fox, 2012. Intra-population variability of life history traits and growth during range expansion of the invasive round goby (*Neogobius melanostomus*). *Fisheries Management and Ecology* 19: 78–88.
- Hudson, P. J., A. P. Dobson & K. D. Lafferty, 2006. Is a healthy ecosystem one that is rich in parasites? *Trends in ecology & evolution* 21: 381–385.
- Huysse, T., V. Audenaert & F. A. M. Volckaert, 2003. Speciation and host–parasite relationships in the parasite genus *Gyrodactylus* (Monogenea, Platyhelminthes) infecting gobies of the genus *Pomatoschistus* (Gobiidae, Teleostei). *International Journal for Parasitology* 33: 1679–1689.
- Huysse, T., M. P. M. Vanhove, M. Mombaerts, F. A. M. Volckaert & H. Verreycken, 2015. Parasite introduction with an invasive goby in Belgium: double trouble? *Parasitology Research* 114: 2789–2793.
- Hyslop, E. J., 1980. Stomach contents analysis – a review of methods and their application. *Journal of Fish Biology* 17: 411–429.
- Höglund, J. & J. Thulin, 1982. Identification of *Diplostomum* spp. in the retina of perch *Perca fluviatilis* and the lens of roach *Rutilus rutilus* from the Baltic Sea – an experimental study. *Systematic Parasitology* 21: 1–19.
- Järv, L., J. Kotta, I. Kotta & T. Raid, 2011. Linking the structure of benthic invertebrate communities and the diet of native and invasive fish species in a brackish water ecosystem. *Annales Zoologici Fennici* 48: 129–141.
- Johnsen, B. O. & A. J. Jensen, 1991. The *Gyrodactylus* story in Norway. *Aquaculture* 98: 289–302.
- Karsiotis, S. I., L. R. Pierce, J. E. Brown & C. A. Stepien, 2012. Salinity tolerance of the invasive round goby: Experimental implications for seawater ballast exchange and spread to North American estuaries. *Journal of Great Lakes Research* 38: 121–128.
- Kelly, D. W., R. A. Paterson, C. R. Townsend, R. Poulin & D. M. Tompkins, 2009. Parasite spillback: a neglected concept in invasion ecology? *Ecology* 90: 2047–2056.
- Kirk, R. S., 2003. The impact of *Anguillicola crassus* on European eels. *Fisheries Management and Ecology* 10: 385–394.
- Koïe, M., 1999. Metazoan parasites of flounder *Platichthys flesus* (L.) along a transect from the southwestern to the northeastern Baltic Sea. *ICES Journal of Marine Science* 56: 157–163.
- Kopp, K. & J. Jokela, 2007. Resistant invaders can convey benefits to native species. *Oikos* 116: 295–301.
- Kornis, M. S., N. Mercado-Silva & M. J. Vander Zanden, 2012. Twenty years of invasion: a review of round goby *Neogobius melanostomus* biology, spread and ecological implications. *Journal of fish biology* 80: 235–285.
- Kornis, M. S., S. Sharma & M. Jake Vander Zanden, 2013. Invasion success and impact of an invasive fish, round goby, in Great Lakes tributaries. *Diversity and Distributions* 19: 184–198.
- Kotta, J., K. Nurkse, R. Puntila & H. Ojaveer, 2016. Shipping and natural environmental conditions determine the distribution of the invasive non-indigenous round goby *Neogobius melanostomus* in a regional sea. *Estuarine, Coastal and Shelf Science* 169: 15–24.
- Kvach, Y., 2001. The round goby parasites in native habitats and in a place of invasion. *Oceanological Studies* 31: 51–57.
- Kvach, Y., 2004a. The Far-Eastern nematode *Anguillicola crassus* – new parasite of the invasive round goby *Neogobius melanostomus* in the Baltic Sea. *Vestnik Zoologii* 38: 38.
- Kvach, Y., 2004b. The metazoa parasites of gobiids in the Dniester Estuary (Black Sea) depending on water salinity. *Oceanological and Hydrobiological Studies* 33: 47–56.
- Kvach, Y., 2005. A comparative analysis of helminth faunas and infection parameters of ten species of Gobiid fishes (actinopterygii: Gobiidae) from the North-Western Black Sea. *Acta Ichthyologica et Piscatoria* 35: 103–110.
- Kvach, Y. & K. E. Skóra, 2006. Metazoa parasites of the invasive round goby *Apollonia melanostoma* (*Neogobius melanostomus*) (Pallas) (Gobiidae: Osteichthyes) in the Gulf of Gdańsk, Baltic Sea, Poland: A comparison with the Black Sea. *Parasitology Research* 100: 767–774.
- Kvach, Y. & C. A. Stepien, 2008. Metazoan Parasites of Introduced Round and Tubenose Gobies in the Great Lakes: Support for the “Enemy Release Hypothesis”. *Journal of Great Lakes Research* 34: 23–35.
- Kvach, Y. & H. M. Winkler, 2011. The colonization of the invasive round goby *Neogobius melanostomus* by parasites

- in new localities in the southwestern Baltic Sea. Parasitology Research 109: 769–780.
- Kvach, Y., Y. Kornychuk, K. Mierzejewska, N. Rubtsova, V. Yurakhno, J. Grabowska & M. Ovcharenko, 2014. Parasitization of invasive gobiids in the eastern part of the Central trans-European corridor of invasion of Ponto-Caspian hydrobionts. Parasitology Research 113: 1605–1624.
- Laudien, J. & M. Wahl, 1999. Indirect effects of epibiosis on host mortality: seastar predation on differently fouled mussels. Marine Ecology 20: 35–47.
- Lymbery, A. J., M. Morine, H. G. Kanani, S. J. Beatty & D. L. Morgan, 2014. Co-invaders: The effects of alien parasites on native hosts. International Journal for Parasitology: Parasites and Wildlife Australian Society for Parasitology 3: 171–177.
- MacInnis, A. J. & L. D. Corkum, 2000. Fecundity and reproductive season of the round goby *Neogobius melanostomus* in the upper Detroit River. Transactions of the American Fisheries Society 129: 136–144.
- Mierzejewska, K., A. Martyniak, T. Kakareko, E. Dzika, K. Stańczak & P. Hliwa, 2011. *Gyrodactylus proterorhini* Ergens, 1967 (Monogeneoidea, Gyrodactylidae) in gobiids from the Vistula River – The first record of the parasite in Poland. Parasitology Research 108: 1147–1151.
- Moravec, F., 1994. Parasitic nematodes of freshwater fishes of Europe. Cluwer Academic Publishers, Dordrecht: 473.
- Ojaveer, H., 2006. The round goby *Neogobius melanostomus* is colonising the NE Baltic Sea. Aquatic Invasions 1: 44–45.
- Ojaveer, H. & J. Kotta, 2015. Ecosystem impacts of the widespread non-indigenous species in the Baltic Sea: literature survey evidences major limitations in knowledge. Hydrobiologia 750: 171–185.
- Ojaveer, H., B. S. Galil, M. Lehtiniemi, M. Christoffersen, S. Clink, A.-B. Florin, P. Gruszka, R. Puntilla & J. W. Behrens, 2015. Twenty five years of invasion: management of the round goby *Neogobius melanostomus* in the Baltic Sea. Management of Biological Invasions 6: 329–339.
- Ondračková, M., K. Francová, M. Dávidová, M. Polačik & P. Jurajda, 2010. Condition status and parasite infection of *Neogobius kessleri* and *N. melanostomus* (Gobiidae) in their native and non-native area of distribution of the Danube River. Ecological Research 25: 857–866.
- Özer, A., 2007. Metazoan parasite fauna of the round goby *Neogobius melanostomus* Pallas, 1811 (Perciformes: Gobiidae) collected from the Black Sea coast at Sinop, Turkey. Journal of Natural History 41: 483–492.
- Palm, H.W., 2011. Fish Parasites as Biological Indicators in a Changing World: Can We Monitor Environmental Impact and Climate Change? H. Mehlhorn (ed), Progress in Parasitology, Parasitology Research Monographs 2
- Peeler, E. J., B. C. Oidtmann, P. M. Midtlyng, L. Miossec & R. E. Gozlan, 2010. Non-native aquatic animal introductions have driven disease emergence in Europe. Biological Invasions 13: 1291–1303.
- Poulin, R., 2002. The evolution of monogenean diversity. International Journal for Parasitology 32: 245–254.
- Prenter, J., C. MacNeil, J. T. A. Dick & A. M. Dunn, 2004. Roles of parasites in animal invasions. Trends in Ecology & Evolution 19: 385–390.
- Rakauskas, V., E. Bacevičius, Ž. Pūtys, L. Ložys & K. Arbačiauskas, 2008. Expansion, Feeding and Parasites of the Round Goby, *Neogobius melanostomus* (Pallas, 1811), a recent invader in the Curonian Lagoon, Lithuania. Acta Zoologica Lituonica 18: 180–190.
- Rakauskas, V., Ž. Pūtys, J. Dainys, J. Lesutienė, L. Ložpys & K. Arbačiauskas, 2013. Increasing Population of the Invader Round Goby, *Neogobius melanostomus* (Actinopterygii: Perciformes: Gobiidae), and its Trophic Role in the Curonian Lagoon, Se Baltic Sea. Acta Ichthyologica Et Piscatoria 43: 95–108.
- Reiczigel, J. & L. Rózsa, 2005. Quantitative Parasitology 3.0. Distributed by the authors, Budapest, <http://www2.univet.hu/qpweb/>.
- Rokicki, J. & L. Rolbiecki, 2002. Colonization of the round goby, *Neogobius melanostomus* (Gobiidae) by parasites in the new environment of the Gulf of Gdansk (Southern Baltic). Wiadomosci Parazytologiczne 48: 197–200.
- Rolbiecki, L., 2006. Parasites of the round goby, *Neogobius melanostomus* (Pallas, 1811), an invasive species in the Polish fauna of the Vistula Lagoon ecosystem. Oceanologia 48: 545–561.
- Rózsa, L., J. Reiczigel & G. Majoros, 2000. Quantifying Parasites in Samples of Hosts. Journal of Parasitology 86: 228–232.
- Ruiz, G. M., P. Fofonoff, A. H. Hines & E. D. Grosholz, 1999. Non-indigenous species as stressors in estuarine and marine communities: Assessing invasion impacts and interactions. Limnology and Oceanography 44: 950–972.
- Sapota, M. R., 2004. The round goby (*Neogobius melanostomus*) in the Gulf of Gdansk – a species introduction into the Baltic Sea. Hydrobiologia 514(219–224): 2004.
- Sapota, M. R., 2011. NOBANIS – Invasive Alien Species Fact Sheet – *Neogobius melanostomus*. Online Database of the European Network on Invasive Alien Species – NOBANIS. www.nobanis.org.
- Sapota, M. R. & K. E. Skora, 2005. Spread of alien (non-indigenous) fish species *Neogobius melanostomus* in the Gulf of Gdansk (south Baltic). Biological Invasions 7: 157–164.
- SAS Institute Inc., 2013. JMP Pro 11.
- Scholz, T., 1999. Life cycles of species of *Proteocephalus*, parasites of fishes in the Palearctic Region: a review. Journal of Helminthology 73: 1–19. doi: [10.1017/S0022149X99000013](https://doi.org/10.1017/S0022149X99000013)
- Skora, K. E. & J. Rzeznik, 2001. Observations on Diet Composition of *Neogobius melanostomus* Pallas 1811 (Gobiidae, Pisces) in the Gulf of Gdansk (Baltic Sea). Journal of Great Lakes Research 27: 290–299.
- Skora, K. E. & J. Stolarski, 1993. New fish species in the Gulf of Gdansk *Neogobius* sp. [cf. *Neogobius melanostomus* (Pallas 1811)]. Bulletin of the Sea Fisheries Institute 1: 83.
- Špakulová, M., M.-J. Perrot-Minnot & B. Neuhaus, 2011. Resurrection of *Pomphorhynchus tereticollis* (Rudolphi, 1809) (Acanthocephala: Pomphorhynchidae) based on new morphological and molecular data. Helminthologia 48: 268–277.
- Szostakowska, B. & H.-P. Fagerholm, 2007. Molecular identification of two strains of third-stage larvae of *Contracaecum rudolphii* sensu lato (Nematoda: Anisakidae) from fish in Poland. The Journal of Parasitology 93: 961–964.

- Szostakowska, B. & H.-P. Fagerholm, 2012. Coexistence and genetic variability of *Contracecaecum rudolphii* A and *Contracecaecum rudolphii* B (Nematoda: Anisakidae) in cormorants, *Phalacrocorax carbo sinensis*, in the Baltic region. *The Journal of Parasitology* 98: 472–478.
- Tabolina, I., 1994. Parasites and diseases of flounder in the coastal waters of Latvia In Bylund, G. & L.-G. Lönnström (eds), Diseases and parasites of flounder in the Baltic Sea. The Baltic Marine Biologists Publication No 15: 61–63.
- Torchin, M. E., K. D. Lafferty, A. P. Dobson, V. J. McKenzie & A. M. Kuris, 2003. Introduced species and their missing parasites. *Nature* 421: 628–630.
- Unger, P., S. Klimpel, T. Lang & H. Palm, 2014. Metazoan parasites from herring (*Clupea harengus* L.) as biological indicators in the Baltic Sea. *Acta Parasitologica* 59: 518–528.
- Ustups, D., U. Bergström, A. B. Florin, E. Kruze, D. Zilniece, D. Elferts, A. Knospina & H. Uzars, 2015. Diet overlap between juvenile flatfish and the invasive round goby in the central Baltic Sea. *Journal of Sea Research* 107: 121–129.
- Valtonen, T. E., 2012. Pääjakso väkäräkärsämadot (Acanthocephala). In Valtonen, T. E., T. Hahalahti-Sirén, A. Karvonen & K. Pulkkinen (eds), Suomen kalojen loiset. Gaudeamus, Tampere.
- Valtonen, T. E. & D. I. Gibson, 1997. Aspects of the biology of diplostomid metacercarial (Digenea) populations occurring in fishes in different localities of Northern Finland. *Annales Zoologici Fennici* 34(1): 47–59.
- van Beek, G. C. W., 2006. The round goby *Neogobius melanostomus* first recorded in the Netherlands. *Aquatic Invasions* 1: 42–43.
- Williams, H. H., K. MacKenzie & A. M. McCarthy, 1992. Parasites as biological indicators of the population biology, migrations, diet, and phylogenetics of fish. *Reviews in Fish Biology and Fisheries* 2: 144–176.
- Zander, C. D., 2003. Four-year monitoring of parasite communities in gobiid fishes of the southwestern Baltic-I. Guild and component community. *Parasitology Research* 90: 502–511.
- Zander, C. D. & L. W. Reimer, 2002. Parasitism at the ecosystem level in the Baltic Sea. *Parasitology* 124: 119–135.

A successful non-native predator, round goby, in the Baltic Sea: generalist feeding strategy, diverse diet and high prey consumption

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Abstract The round goby *Neogobius melanostomus* has successfully invaded much of the Baltic Sea. However, very little is known about the feeding habits of the species in this newly invaded environment. Our laboratory experiment showed that the round goby is able to effectively consume a diverse variety of prey when given the choice between dominant benthic invertebrates: bivalves (*Macoma balthica*, *Mytilus trossulus*, *Cerastoderma glaucum*) and amphipods (*Gammarus* spp.). In contrast consumption of the gastropod (*Theodoxus fluviatilis*) was very low in all provided combinations. Nevertheless, the round goby had no statistically significant preference towards any of the prey taxa. The round goby exhibited size-specific consumption of *M. trossulus*, with smaller individuals being consumed at least 25% more than larger size classes. In addition elevated prey density resulted in higher consumption of prey by the fish. The broad diet suggests that shifting densities of benthic invertebrate prey has little influence on the further dispersal of the round goby in the Baltic Sea as the species is potentially able to switch between several native invertebrate taxa. This opportunistic feeding behaviour has likely favoured this invasion and

ensured success of the species in the invaded ecosystem.

Keywords Baltic Sea · Benthic invertebrate prey · Consumption rates · Generalist · Prey preference

Introduction

In recent decades, growing numbers of non-indigenous species have significantly influenced biodiversity and dominance structure of benthic communities in coastal habitats (Olenin & Leppäkoski, 1999; Leppäkoski & Olenin, 2000; Bax et al., 2003; Strayer et al., 2006). Non-indigenous species may alter food web dynamics and functioning in the local ecosystem through intensified predation pressure on native species, or by targeting different prey items than native predators (Zavaleta et al., 2001; Laxson et al., 2003). The success and impact of non-native species are partly determined by their feeding strategy and selectivity. A generalist feeding strategy is less affected by shifting prey availability while a specialist feeding strategy could result in fluctuating population abundance of the predator when preferred prey abundance varies (Volterra, 1928). In conditions where prey biomass is not a limiting factor and top predators are absent, a generalist predator could have a more severe effect on communities that they prey upon (Schreiber, 1997).

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In the brackish waters of the north-eastern Baltic Sea, predatory pressure on benthic communities is rather weak (Kautsky, 1981), represented by a few molluscivorous benthic fishes such as flounder *Platichthys flesus* (Linnaeus, 1758) and viviparous eelpout.

Zoarces viviparus (Linnaeus, 1758) (Ojaveer et al., 2010). The invasion of the round goby *Neogobius melanostomus* (Pallas 1811) in the early 2000s changed the situation dramatically through notable intensification of top-down control of benthic invertebrates (Järv et al., 2011). High feeding rates and increasing abundance potentially allows the round goby to substantially change the richness and dominance structure of benthic communities (Lederer et al., 2008; Raby et al., 2010; Kornis et al., 2012; Rakauskas et al., 2013).

In order to be a successful invader, a species must be able to adapt to novel environmental conditions that include the available prey. Based on diet studies, the round goby feeds excessively on bivalves, when present, but can also consume other types of food, with a great flexibility in terms of prey species (Corkum et al., 2004; Barton et al., 2005; Kornis et al., 2012). In its native range, in the Ponto-Caspian region, the round goby mainly preys on bivalves (*Cerastoderma* spp., *Mya* spp., *Mytilus* spp.), but gastropods (*Hydrobia* spp.), worms, Gammaridae, fish (sprat *Sprattus sprattus* (Linnaeus, 1758)) and detritus also play an important role in their diet throughout the year (Skazhkina & Kostyuchenko, 1968). In invaded North American rivers, the goby feeds extensively on non-native dreissenid mussels (*Dreissena polymorpha* (Pallas, 1771) and *Dreissena bugensis* (Andrusov, 1897)) when these mussels are present (Raby et al., 2010). Dreissenids are absent from many North American rivers invaded by goby (Carman et al., 2006; Kornis & Vander Zanden, 2010), and in such systems gobies feed primarily on a variety of insect larvae species, but not on native bivalves (Ghedotti et al., 1995; Carman et al., 2006; Kornis et al., 2012). In some Baltic Sea areas, the round goby feeds primarily on *Mytilus trossulus* Gould, 1850 (Skora & Rzeznik, 2001), while in others decapods dominate in the diet (Azour, 2011). In the northern Baltic Sea bivalves serve as the main food source of the round goby and are the dominant benthic invertebrate taxa in the coastal sea (Norling & Kautsky, 2008; Järv et al., 2011).

Obtaining information on species dietary preference is important because selectivity toward some prey species may cause an uneven predatory pressure within a benthic community, and could ultimately modify both community size structure and species composition (Post & Cucin, 1984). The round goby has already shown in other invaded areas that it can significantly lower abundances of dominant prey species (Lederer et al., 2008; Raby et al., 2010). Knowing the consumption rates and preferences of different prey species is key in assessing the impact of the round goby on the size and distribution range of prey species populations. In addition, feeding experiments provide understanding on the round goby's plasticity in terms of prey consumption and therefore assist in evaluating its likely impacts on invaded habitats. Most of the knowledge about the diet of round goby has been obtained from gut analyses (Ray & Corkum, 1997; Skora & Rzeznik, 2001), which may reflect more on prey availability than preference towards particular prey species (Underwood et al., 2004). Estimating prey preference from stomach content analysis is only possible when fine-scale prey availability and predator abundance in the area is known. In the north-eastern Baltic Sea only estimates on average prey density are available for very heterogeneous areas and there are no published values of round goby density. Thus, feeding experiments can offer the opportunity to determine prey preference under controlled conditions. In North America, several studies on the size selection of dreissenid mussels by the round goby showed that the consumption of different-sized mussels is related to the size of the round goby with larger gobies preferring larger mussels (Ghedotti et al., 1995; Andraso et al., 2011). Similar studies have not been conducted in the north-eastern Baltic Sea.

The primary aim of the current study was to experimentally demonstrate whether the round goby has a selective feeding preference among several taxa of locally dominating benthic invertebrates in the north-eastern Baltic Sea. In determining the dietary preference of the species, the following secondary research questions were addressed: (1) are some prey types and sizes consumed more than others, and (2) are prey taxa consumed at a higher rate when provided at higher densities? In addition the used densities reflect naturally low and naturally high prey density and therefore show if the round goby is able to impact

benthic invertebrate communities even at seasonal abundance peaks.

Materials and methods

Study area

The indoor laboratory experiment was conducted in August 2014 at Kõiguste field station, located in the north-eastern Baltic Sea, in Estonia, on the northern shore of Gulf of Riga (58°22′23.5″N; 22°58′56.3″E). In the laboratory experiment, we created environmental conditions and prey density similar to those in this region of the Baltic Sea. Generally the area is quite shallow with prevailing depths between 1 and 4 metres and a low salinity of 4.0–6.5 psu. The prevailing substrate type is a thin layer of slightly silted sand mixed with pebbles, gravel and boulders. The area has high nutrient levels and habitat diversity, providing abundant algal and invertebrate communities (Kotta et al., 2008). In order to create similar conditions in all aquaria, the complexity of habitat was reduced. Artificial plants provided shelter for amphipods and gastropods, a hollow artificial structure provided shelter for round goby and was an attachment substrate for mussels, and sand provided natural habitat for clams.

Test organisms

All prey animals were collected adjacent to the field station by a landing net or by a scuba diver, and let to acclimate for 12 h in the aquaria before the experiment. Five key invertebrate taxa typical for the north-eastern Baltic Sea (Lauringson & Kotta, 2006) were used as prey: the bivalves *Mytilus trossulus*, *Macoma balthica* (Linnaeus, 1758) and *Cerastoderma glaucum* (Bruguère, 1789); the gastropod *Theodoxus fluviatilis* (Linnaeus, 1758); and gammarid amphipods (mainly *Gammarus tigrinus* Sexton, 1939, *Gammarus salinus* Spooner, 1947, *Gammarus zaddachi* Sexton, 1912, *Gammarus oceanicus* Segerstråle, 1947). Because of their significant size variation in space and time, *M. balthica* and *M. trossulus* were divided into different size classes. Invertebrate densities (Table 1) reflected their natural densities in the area. Two density levels were used in various treatments to replicate summer (high) and spring (low) density

conditions (Kotta et al., 2008; Veber et al., 2009; Martin et al., 2013).

Round goby (33 individuals) were collected live from a local fishermen's basket trap (5 m depth) on 2nd of August 2014, adjacent to the field station, and were kept in separate gently aerated aquaria with no food for 24 h before both runs (see sections below) of the experiment. The goby varied in size (mean total length 131 mm, range 104–177 mm) and sex (67% male). The selected fish represented a similar size frequency distribution to adult round goby in the field, except that juveniles were not used, as the round goby undertakes an ontogenetic shift in its diet (Ray & Corkum, 1997, 2001). Spawning behaviour likely did not affect food consumption of goby during the experiment as only males stop feeding when guarding nests (Corkum et al., 1998), and nests were not present in the aquaria.

Experimental setup

The experiment was conducted in thirty-three 50 l aquaria (bottom surface area 0.11 m²) in a temperature-controlled room (21°C). The bottom of each aquarium was covered with a 30 mm layer of sand and filled with 40 l of seawater. An empty medium-sized flower pot ($r_{\text{bottom}} = 50$ mm, $r_{\text{top}} = 35$ mm, $h = 100$ mm) and a plastic plant (external surface area 0.06 m²), together with an aerator tube were placed in each aquarium. Sand and seawater were collected adjacent to the experimental site. In order to remove excessive animal and plant material, sand was sundried for 3 days and then sieved through a 1 mm mesh net prior to the experiment. Light intensity (17:7 h L:D) and photoperiod were similar to the local ambient environment in August.

The design of the experiment follows the suggestions of Underwood & Clarke (2005) and subsequent amendments by others (Manly, 2006; Underwood & Clarke, 2006, 2007; Taplin, 2007). As our aim was to determine the feeding rates of the round goby under conditions resembling the field values, the studied prey taxa had different initial densities (Table 1). The design included two stages for prey preference calculations (Table 1): each prey taxon separately at high density conditions (hereafter no choice high density) and all prey taxa together at low density conditions (hereafter choice). Prey preference is indicated when the relative consumption of prey taxon is significantly

Table 1 Counts of prey taxa used in different experimental treatments in a 40 l aquarium: 1–5 no choice high density (stage 1), 6 choice (stage 2), and 7–11 no choice low density (stage 3)

Stage	1	1	1	1	1	2	3	3	3	3	3
Treatment no	1	2	3	4	5	6	7	8	9	10	11
Taxon*											
<i>M. trossulus</i> total	124					40	40				
Small ($3 \leq \dots \leq 12$)	79					24	24				
Medium ($12 \leq \dots \leq 20$)	39					12	12				
Large ($20 \leq \dots \leq 30$)	6					4	4				
<i>M. balthica</i> total		110				24		24			
Small ($3 \leq \dots \leq 7$)		39				15		15			
Medium ($7 \leq \dots \leq 12$)		65				7		7			
Large ($12 \leq \dots \leq 16$)		6				2		2			
<i>C. glaucum</i> ($7 \leq \dots \leq 12$)			90			5			5		
<i>T. fluviatilis</i> ($5 \leq \dots \leq 7$)				90		25				25	
<i>Gammarus</i> spp. ($5 \leq \dots \leq 8$)					90	30					30
No of replicates	4	6	6	6	6	6	6	6	6	6	6

Each treatment used different 40 l aquaria. Numbers in brackets represent the total length (TL) of individuals in mm. Half of the replicates for all treatments were conducted during the first run and half during the second run

* Bivalves *Mytilus trossulus*, *Macoma balthica*, *Cerastoderma glaucum*, gastropod *Theodoxus fluviatilis* and amphipods *Gammarus* spp

** Numbers in bold represent total counts per taxon

higher in choice conditions compared to no choice high density conditions. We also included a third stage with all prey taxa provided separately and at low density (hereafter no choice low density). When combined with no choice high density (Stage 1), Stage 3 allowed us to assess if the predation of round goby depends on the density of its prey.

A combination of 5 taxa and 3 stages resulted in 11 different treatments (Table 1): no choice high density (altogether 5 treatments, one for each taxon), choice (1 treatment including all taxa), and no choice low density (altogether 5 treatments, one for each taxon). All treatments were replicated 6 times. As an exception, the *M. trossulus* treatment under no choice high density had only 4 replicates. A separate one-way permutational ANOVA analysis showed that the size of the fish did not significantly differ among experimental treatments ($F_{10,53} = 1.658$; $P = 0.1158$).

Most aquaria were used twice. Half of the replicate aquaria of each treatment (see also section above and Table 1) were used in the first run (3 replicates \times 11 treatments = 33) and the other half were used in the second run (31). One round goby was used per aquarium. Before the second run, fish specimens were

randomly reassigned to aquaria and no goby was used for the same treatment twice. The two runs were conducted 24 h apart. Before the second run the aquaria were set up with new seawater, sediment, plastic plant, flower pot and prey species.

The experiment was run for 16 h from 10 pm until 2 pm, allowing the fish to forage during total darkness and at dawn, a period when round goby typically feed (Karlson et al., 2007 and references therein). In addition, five control aquaria without the round goby were established; in all five aquaria all the prey animals were retrieved alive after the experiment.

Post experimental invertebrate and fish handling

After the experiment, the round goby's total length was measured to the nearest 0.01 mm and sex was determined through the shape of the urogenital papilla (Charlebois et al., 1997). Sediment with invertebrates was kept in a freezer at -20°C and the remaining prey animals were separated under a binocular microscope from the sediment in the laboratory. Invertebrates were determined to a species level, counted, and their dry weight (dw) was measured to the nearest 0.0001 g

after 48 h in a 60°C drying oven. All prey individuals were measured before and after the experiment with an electronic calliper to the nearest 0.01 mm. An additional 20 prey individuals for each taxon and size class served as control to obtain the taxon-specific length-weight relationships and these regression equations were used to calculate the dry weights of consumed prey individuals.

Data analyses

Statistical model

Permutational tests for factorial ANOVA/ANCOVA design were used in order to compare absolute prey consumption, to assess prey preferences and to investigate size-specific predation of bivalves among the studied factors and treatment levels as well as to assess if predation by the round goby is density dependent (see below sections). Permutational ANOVA/ANCOVA does not assume the data to have any specific distributions. In order to investigate differences between treatment levels, pair-wise comparisons among all pairs of levels of a given factors of interest were obtained by using pseudo-t statistic, a multivariate analogue of the univariate-t statistic. Package Vegan (Oksanen et al., 2015) in the R environment was used for the analyses and all analyses were based on Euclidean distances of the original raw data.

We initially assumed that fish size had significant effects on various metrics of prey consumption and therefore our initial statistical design involved permutational ANCOVA analyses (covariate: fish total length). However, as the effect of the covariate was not statistically significant in any of the statistical tests (all $P > 0.20$), permutational ANOVA analyses without covariate were run and thereby only these results are reported below.

Prey taxa preference

Permutational ANOVA/ANCOVA analyses were used to investigate prey preferences by the round goby. Dependent variables were the relative consumptions of prey in count and in biomass, with prey taxa (*M. trossulus*, *M. balthica*, *C. glaucum*, *T. fluviatilis* and *Gammarus* spp.) and choice (no choice high

density, choice) as factors. Relative consumptions were calculated according to Taplin (2007):

$$p_i = a_i \div \sum_{i=1}^k a \quad \text{and} \quad q_i = b_i \div \sum_{i=1}^k b,$$

where p_i is the ratio of consumptions of each prey taxon ($i = 1, \dots, 5$) in no choice high density stage (a_i) to total prey consumption in no choice high density stage; and q_i is the ratio of consumptions of each prey taxon ($i = 1, \dots, 5$) in the choice stage (b_i) to total prey consumption in choice stage. In choice conditions, a taxon's relative consumption was calculated on the basis of one aquarium (replicated 6 times). Since in no choice high density conditions all species were offered separately, relative consumption was calculated on a basis of randomly assigned groups of all 5 taxa (replicated 6 times).

Size-specific consumption

Permutational ANOVA/ANCOVA analyses were used to investigate size-specific predation of bivalves by the round goby with prey availability (no choice low density, no choice high density and choice) and size (small, medium, large) as factors. The dependent variables included consumed relative count and relative biomass of each of the common bivalves (*M. trossulus* or *M. balthica*). The relative consumption refers to the proportions of consumed prey size to the availability of prey in the same size. Calculations were done for each prey taxon size in no choice low density, no choice high density and choice stage.

Density-dependent predation

Permutational ANOVA/ANCOVA analyses were used to assess if predation by the round goby depends on prey density. In this analysis factors included prey density (no choice low density, no choice high density) and taxa (*M. balthica*, *M. trossulus*, *C. glaucum*, *Gammarus* spp., *T. fluviatilis*). The dependent variables were absolute and relative consumption of either count or biomass of prey. The relative consumption refers to the proportions of consumed prey taxon to the availability of the same prey taxon. Calculations were done for each prey taxon in no choice low density and no choice high density stage.

Results

The round goby consumed all bivalve species (*M. trossulus*, *M. balthica*, *C. glaucum*) and *Gammarus* spp. The gastropod *T. fluviatilis* was generally avoided with their relative consumption by count remaining below 3%. During the 16 h experimental period, each round goby consumed on average 0.99 ± 0.22 g dw when mixed diet was available and 2.28 ± 0.81 g dw when a single taxon at high density was available.

Prey taxa preference

The round goby showed no preference towards any of the studied taxa, since relative consumptions of each prey taxon did not differ between no choice (p_i) and choice (q_i) conditions (Table 2). Although the round

goby showed no prey preference towards prey taxa, there were differences in consumption between taxa, with some taxa being consumed more than others (Fig. 1; Table 2). The differences between taxa were different under no choice conditions compared to choice conditions, as reflected by the significant interaction effect (Table 2).

Size-specific predation of bivalves

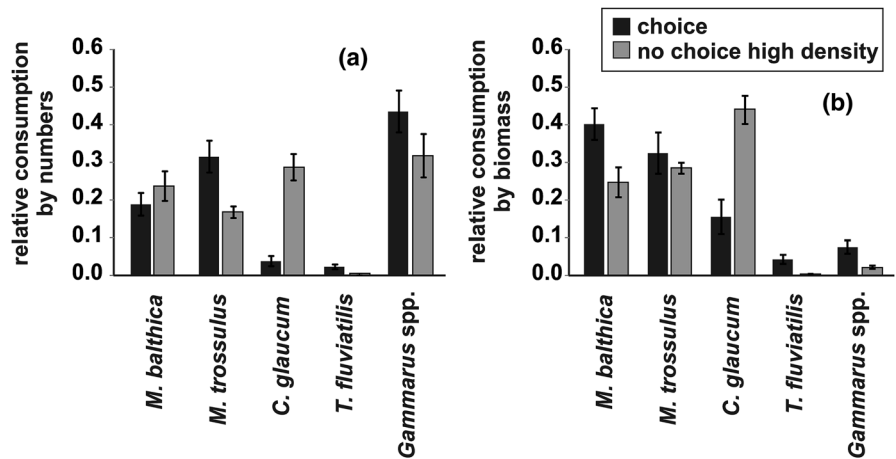
The size of bivalves affected the predation rate of round goby and the responses were species specific. There was no statistical evidence of size selection in the relative consumption of *M. balthica* in count between no choice high density, no choice low density or choice stages or within stages (Table 2). In contrast, round goby consumed more small-sized *M. trossulus* over medium-

Table 2 Statistics from Permutational ANOVA analyses for prey consumption by the round goby

Analysis	Dependent variable	Factors	df	F	P
Prey taxa preference					
	Relative count	Choice	1, 50	0.01	0.91
		Taxon	4, 50	18.85	0.001
		Choice × taxon	4, 50	6.82	0.001
	Relative biomass	Choice	1, 50	0.001	0.99
		Taxon	4, 50	30.71	0.001
		Choice × taxon	4, 50	9.52	0.001
Size-specific predation of bivalves					
	Relative count of <i>M. balthica</i>	Prey availability	2, 45	0.70	0.47
		Prey size	2, 45	1.53	0.23
		Prey availability × density	4, 45	0.50	0.77
	Relative count of <i>M. trossulus</i>	Prey availability	2, 39	0.75	0.51
		Prey size	2, 39	19.12	0.001
		Prey availability × density	4, 39	1.13	0.37
Density-dependent predation					
	Absolute count	Prey density	1, 48	39.40	0.001
		Prey taxon	4, 48	10.16	0.001
		Density × taxon	4, 48	3.89	0.01
	Absolute biomass	Prey density	1, 48	68.15	0.001
		Prey taxon	4, 48	22.27	0.001
		Density × taxon	4, 48	16.63	0.001
	Relative count	Prey density	1, 48	0.11	0.76
		Prey taxon	4, 48	12.16	0.001
		Density × taxon	4, 48	0.69	0.63
	Relative biomass	Prey density	1, 48	0.29	0.62
		Prey taxon	4, 48	17.49	0.001
		Density × taxon	4, 48	2.03	0.10

* Numbers in bold represent statistically significant p -values

Fig. 1 The relative consumption of five prey taxa by the round goby in terms of prey numbers (a) and biomass (b) (g) during 16 h (mean ± SE). Relative consumption refers the ratio of consumption of each prey taxon in no choice high density stage (or choice stage) to total prey consumption in no choice high density stage (or choice stage) (Taplin, 2007). In general, each treatment was replicated 6 times



sized *M. trossulus* in relative count under choice conditions ($P = 0.006$) and more than large-sized *M. trossulus* under no choice low density conditions ($P = 0.007$) and choice conditions ($P = 0.006$) (Table 2).

Density-dependent predation

The round goby exhibited density-dependent consumption on the investigated prey taxa. In general, the absolute consumption of each prey group increased

with increased prey density (Fig. 2). The absolute consumption in both count and biomass depended on prey density, prey taxon and the interaction between prey taxon and density (Table 2). Doubling of prey density led to an average twofold increase in the feeding rate for the three bivalve species and an average 1.5 times increase for *Gammarus spp.* No such patterns were observed for the relative consumption (Fig. 1). The relative consumption both in count and in biomass depended only on prey taxon (Table 2).

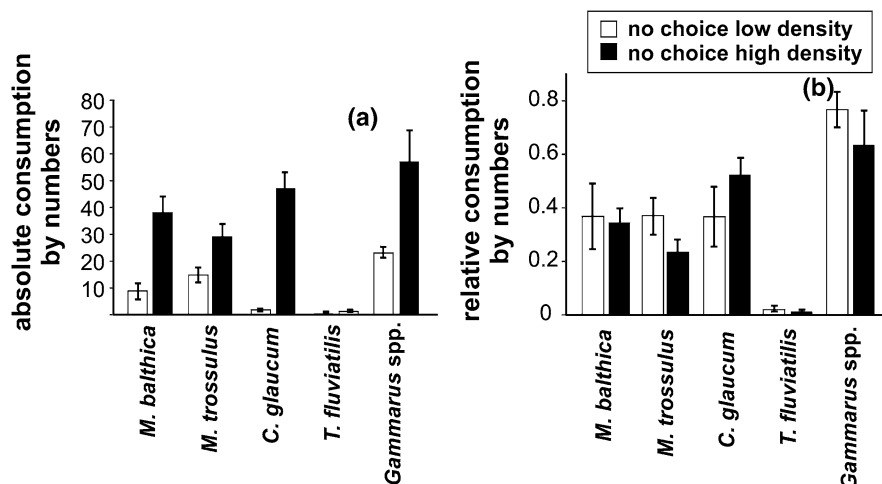


Fig. 2 Absolute (a) or relative (b) consumption of five prey taxa by the round goby (mean ± SE) in terms of count (ind per 16 h) of prey. The relative consumption refers to the proportions of consumed prey taxon to the availability of the same prey taxon in the same aquarium (compared to Fig. 1a, where relative

consumption is calculated according to Taplin, 2007). Calculations were done for each prey taxon in no choice low density and no choice high density stage. In general, each treatment was replicated 6 times

Discussion

The key finding of the study is that the round goby does not select for any of the studied key invertebrate taxa in the north-eastern Baltic Sea. This result indicates that this invasive non-indigenous species is a generalist in its diet, as has been shown also in a previous study (Karlson et al., 2007). The round goby consumed most of the available native taxa, similar to results observed in previous studies from North America (Brandner et al., 2013). Together, these results suggest round goby is a feeding generalist, a trait that should favour successful invasion (Ribeiro et al., 2007; Brandner et al., 2013). Earlier investigations indicated the round goby preying more on some prey species than others (Barton et al., 2005). Nevertheless, in the light of the current study, this result cannot be attributed to preference, as such, but rather to the consumption of the dominant species in the benthic realm. This finding has important ecological implications, as with the removal of dominant benthic invertebrate species the round goby may switch to subdominant taxa. Such prey switching may ultimately affect a broad range of benthic communities (including their functions), unless the production of invertebrates exceeds consumption by this invasive fish species, as has been seen in some areas (Kornis et al., 2013).

Some benthic invertebrates such as the gastropod *T. fluviatilis*, that was practically not consumed, could benefit from the presence of round goby. Such protected species may increase their abundance due to declined competition for food resources and habitat. Thus, the consequence of the invasion of the round goby could be strictly context dependent, as evidenced for other non-native species in other invaded ecosystems (e.g. de Moura Queirós et al., 2011; Barrios O'Neill et al., 2014).

The Baltic Sea provides suitable abiotic and biotic conditions for the round goby (Sapota, 2004; Sokołowska & Fey, 2011; Kornis et al., 2012). Our results confirm that the round goby is a generalist feeder capable of consuming a wide spectrum of invertebrate prey, which increases its invasion potential to yet uncolonised areas in the Baltic Sea. The intraspecific aggression previously observed in round goby (Groen et al., 2012) may prevent total depletion of prey as the larger territorial goby force smaller individuals and juveniles to migrate out of good feeding/sheltering grounds (Charlebois et al., 2001).

This experimental study showed the round goby to be a very effective predator and appeared to consume diverse prey at high rates (up to 0.99 g dw per 16 h for a mixed species diet). Since consumption rates of the round goby have not previously been experimentally investigated in the Baltic Sea (Ojaveer & Kotta, 2015), comparisons can only be made with experiments from other areas. Thus, all comparisons must be interpreted with the assumption that the fish were provided with different prey amounts and species and water temperature and round goby size could be substantially different. In general the consumption rates in this study were higher than other areas. In the Laurentian Great Lakes, the bivalve *D. polymorpha* was consumed at a rate of 1 g (max 6.5) wet weight (ww) daily (Ray & Corkum, 1997) and *D. polymorpha* with amphipods up to 0.022 g shell-free dw h⁻¹ (corresponding to 0.62 g dw daily, according to Diggins et al., 2002; Jurkiewicz-Karnkowska, 2005). Moreover, laboratory experiments showed that the round goby consumes native clams of the Great Lakes even at lower rates (an average of 4 times less than zebra mussels) (Ghedotti et al., 1995). Taking into account smaller size of the fish in North American populations compared to the Baltic Sea (Sokołowska & Fey, 2011; Kornis et al., 2012), these published values are still considered several times lower than observed in the current experiment.

Round goby is a territorial fish with estimated home range of 5 m² (Ray & Corkum, 2001). While densities of this species are yet to be determined in the Baltic Sea, the values range from 0.3 to 9 individuals m⁻² in the Great Lakes (Ray & Corkum, 2001). If the round goby achieves similar densities in the coastal area of the Baltic Sea and prey consumption rates are similar to those observed in our experiment, then providing an average benthic biomass of 50 g dw m⁻² (Kotta et al., 2009), the round goby could deplete local benthic invertebrate communities in a very short time (within 10 days). Since benthic biomass and fish densities are patchy (Kotta et al. 2015, 2016), such depletions may be localised. In a laboratory experiment the feeding rates are expected to be significantly higher than in nature due to easier foraging for prey, reduced availability of refuges for prey, and reduced overall habitat complexity. Furthermore, in the field during breeding seasons feeding rates of round goby are likely lower due to aggression among males and nest guarding behaviour (Helfman, 1986; Ray & Corkum,

2001; Belanger & Corkum, 2003). In addition, the presence of other predatory fish may further reduce feeding activity of the round goby (Marentette & Balshine, 2012). Nevertheless, the consumed amounts are still high enough to demonstrate that the estimated predation rates of the round goby are much higher than secondary productivity by benthic invertebrates (e.g. Westerbom et al., 2002). Thus, the round goby has the potential to affect benthic communities drastically. However, such severe impacts have not yet been documented in the Baltic Sea, partly because of a lack of such impact studies (e.g. Ojaveer & Kotta, 2015) and round goby may have much lower densities with patchy distributions.

The current study indicated that the relative consumption by the round goby did not vary among the size classes of *M. balthica*; however, the relative consumption of small *M. trossulus* individuals (average 46%) was higher than medium (average 17%) or large (average 3%) individuals. In order to show true size-specific preference, additional experiments with consumption measured separately for each shell size class, are needed (Underwood & Clarke, 2005; Taplin, 2007).

The round goby also exhibited density-dependent feeding behaviour with elevated consumption rates at no choice high prey densities. Such behaviour is also common in other epibenthic species (Mansour & Lipcius, 1991). The density-dependent functional response could be a result of longer foraging time at low prey densities (MacArthur & Pianka, 1966) in which less prey are handled per unit time compared to high prey densities. Alternatively, low abundance allowed mobile prey species to find shelter, as availability of shelter in boulders or gravel has shown to decrease the consumption rates of amphipods by predators even with low availability of shelter (Diehl, 1992; Diggins et al., 2002). Although the absolute consumptions of prey taxa were higher at high prey density, the relative consumptions were similar between low and high prey density levels. Higher absolute consumption shows that the more there is, the more is eaten. The relative consumption rates, however, suggest similar impacts of the round goby to community structure. In field foraging animals are expected to distribute themselves among patches of prey of varying density in a way that the average foraging success for predators is equal in all patches (Kacelnik et al., 1992).

In conclusion, our study suggests that the round goby is capable of severely impacting native benthic invertebrate populations and thereby causing multiple impacts in the coastal ecosystems of the Baltic Sea. A generalist feeding strategy, coupled with exceptionally high consumption rates may result in the increase of the round goby population until significant reductions in the abundance of prey populations take place. Intraspecific competition for food and habitat, multiple physical disturbances and predation by native fish may potentially stabilise the abundance of the round goby in future. In North America, in the Lake Erie burbot *Lotalota* (Linnaeus, 1758) likely controls the abundance of the round goby (Madenjian et al., 2011). To date, however, in the Estonian coastal range (and potentially elsewhere in the Baltic Sea) local predatory fish (perch *Perca fluviatilis* Linnaeus, 1758, zander *Sander lucioperca* (Linnaeus, 1758), northern pike *Esox lucius* Linnaeus, 1758) have not yet been shown to significantly reduce the round goby population (database of the Estonian Marine Institute).

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Compliance with Ethical Standards

Animal rights Estonian Ethics Commission granted approval (Permission Nr 35/2014) for the use of animals in the research.

References

- Andraso, G. M., M. T. Ganger & J. Adamczyk, 2011. Size-selective predation by round gobies (*Neogobius melanostomus*) on dreissenid mussels in the field. *Journal of Great Lakes Research* 37: 298–304.
- Azour, F., 2011. Fødebiologi hos den sortmundede kutling *Neogobius melanostomus* i danske farvande. http://fiskeatlas.ku.dk/billeder/Sortmundet_kutlings_f_debiologi_i_DK.pdf.
- Barrios O'Neill, D., J. T. A. Dick, A. Ricciardi, H. J. MacIsaac & M. C. Emmerson, 2014. Deep impact: in situ functional responses reveal context-dependent interactions between vertically migrating invasive and native mesopredators and shared prey. *Freshwater Biology* 59: 2194–2203.
- Barton, D. R., R. A. Johnson, L. Campbell, J. Petruniak & M. Patterson, 2005. Effects of round gobies (*Neogobius*

- melanostomus*) on dreissenid mussels and other invertebrates in Eastern Lake Erie, 2002–2004. *Journal of Great Lakes Research* 31: 252–261.
- Bax, N., A. Williamson, M. Aguero, E. Gonzalez & W. Geeves, 2003. Marine invasive alien species: a threat to global biodiversity. *Marine Policy* 27: 313–323.
- Belanger, R. M. & L. D. Corkum, 2003. Susceptibility of tethered round gobies (*Neogobius melanostomus*) to predation in habitats with and without shelters. *Journal of Great Lakes Research* 29: 588–593.
- Brandner, J., J. Pander, M. Mueller, A. F. Cerwenka & J. Geist, 2013. Effects of sampling techniques on population assessment of invasive round goby *Neogobius melanostomus*. *Journal of Fish Biology* 82: 2063–2079.
- Carman, S. M., J. Janssen, D. J. Jude & M. B. Berg, 2006. Diel interactions between prey behaviour and feeding in an invasive fish, the round goby, in a North American river. *Freshwater Biology* 51: 742–755.
- Charlebois, P. M., J. E. Marsden, R. G. Goettel, R. K. Wolfe, D. J. Jude & S. Rudnika, 1997. The Round Goby, *Neogobius melanostomus* (Pallas), A Review of European and North American Literature. Illinois-Indiana Sea Grant Program and Illinois Natural History Survey, Zion.
- Charlebois, P. M., L. D. Corkum, D. J. Jude & C. Knight, 2001. The round goby (*Neogobius melanostomus*) invasion: current research and future needs. *Journal of Great Lakes Research* 27: 263–266.
- Corkum, L. D., A. J. Macinnis & R. G. Wickett, 1998. Reproductive habits of round gobies. *Great Lakes Research Review* 3: 13–20.
- Corkum, L. D., M. R. Sapota & K. E. Skora, 2004. The round goby, *Neogobius melanostomus*, a fish invader on both sides of the Atlantic Ocean. *Biological Invasions* 6: 173–181.
- De Moura Queirós, A., J. G. Hiddink, G. Johnson, H. N. Cabral & M. J. Kaiser, 2011. Context dependence of marine ecosystem engineer invasion impacts on benthic ecosystem functioning. *Biological Invasions* 13: 1059–1075.
- Diehl, S., 1992. Fish predation and benthic community structure: the role of omnivory and habitat complexity. *Ecology* 73: 1646–1661.
- Diggins, T. P., J. Kaur, R. K. Chakraborti & J. V. DePinto, 2002. Diet choice by the exotic round goby (*Neogobius melanostomus*) as influenced by prey motility and environmental complexity. *Journal of Great Lakes Research* 28: 411–420.
- Ghedotti, M. J., J. C. Smihula & G. R. Smith, 1995. Zebra mussel predation by round gobies in the laboratory. *Journal of Great Lakes Research* 21: 665–669.
- Groen, M., N. M. Sopinka, J. R. Marentette, A. R. Reddon, J. W. Brownscombe, M. G. Fox, S. E. Marsh-Rollo & S. Balshine, 2012. Is there a role for aggression in round goby invasion fronts? *Behaviour* 149: 685–703.
- Helfman, G. S., 1986. Fish behaviour by day, night and twilight. In Pitcher, T. J. (ed.), *The Behaviour of Teleost Fishes*. Springer, US: 366–387.
- Jurkiewicz-Karnkowska, E., 2005. Some aspects of nitrogen, carbon and calcium accumulation in molluscs from the Zegrzynski reservoir ecosystem. *Polish Journal of Environmental Studies* 14: 173–177.
- Järv, L., J. Kotta, I. Kotta & T. Raid, 2011. Linking the structure of benthic invertebrate communities and the diet of native and invasive fish species in a brackish water ecosystem. *Annales Zoologici Fennici* 48: 129–141.
- Kacelnik, A., J. R. Krebs & C. Bernstein, 1992. The ideal free distribution and predator-prey populations. *Trends in Ecology & Evolution* 7: 50–55.
- Karlson, A. M. L., G. Almqvist, K. E. Skóra & M. Appelberg, 2007. Indications of competition between non-indigenous round goby and native flounder in the Baltic Sea. *ICES Journal of Marine Science* 64: 479–486.
- Kautsky, N., 1981. On the trophic role of the blue mussel (*Mytilus edulis* L.) in the Baltic coastal ecosystem and the fate of the organic matter produced by the mussels. *Kieler Meeresforsch* 5: 454–461.
- Kornis, M. S. & M. J. Vander Zanden, 2010. Forecasting the distribution of the invasive round goby (*Neogobius melanostomus*) in Wisconsin tributaries to Lake Michigan. *Canadian Journal of Fisheries and Aquatic Sciences* 67: 553–562.
- Kornis, M. S., N. Mercado-Silva & M. J. Vander Zanden, 2012. Twenty years of invasion: A review of round goby *Neogobius melanostomus* biology, spread and ecological implications. *Journal of Fish Biology* 80: 235–285.
- Kornis, M. S., S. Sharma & M. J. Vander Zanden, 2013. Invasion success and impact of an invasive fish, round goby, in Great Lakes tributaries. *Diversity and Distributions* 19: 184–198.
- Kotta, J., V. Lauringson, G. Martin, M. Simm, I. Kotta, K. Herkül & H. Ojaveer, 2008. Gulf of Riga and Pärnu Bay. In Schiewer, U. (ed.), *Ecology of Baltic Coastal Waters*. Springer, Berlin: 217–243.
- Kotta, J., I. Kotta, M. Simm & M. Pöllupüü, 2009. Separate and interactive effects of eutrophication and climate variables on the ecosystem elements of the Gulf of Riga. *Estuarine, Coastal and Shelf Science* 84: 509–518.
- Kotta, J., K. Oganjan, V. Lauringson, M. Pärnoja, A. Kaasik, L. Rohtla, I. Kotta & H. Orav-Kotta, 2015. Establishing functional relationships between abiotic environment, macrophyte coverage, resource gradients and the distribution of *Mytilus trossulus* in a brackish non-tidal environment. *PLoS ONE* 10: e0136949.
- Kotta, J., K. Nurkse, R. Puntila & H. Ojaveer, 2016. Shipping and natural environmental conditions determine the distribution of the invasive non-indigenous round goby *Neogobius melanostomus* in a regional sea. *Estuarine Coastal and Shelf Science* 169: 15–24.
- Lauringson, V. & J. Kotta, 2006. Influence of the thin drift algal mats on the distribution of macrozoobenthos in Kõiguste Bay, NE Baltic Sea. *Hydrobiologia* 554: 97–105.
- Laxson, C. L., K. N. McPhedran, J. C. Makarewicz, I. Telesh & H. J. Macisaac, 2003. Effects of the non-indigenous cladoceran *Cercopagis pengoi* on the lower food web of Lake Ontario. *Freshwater Biology* 48: 2094–2106.
- Lederer, A. M., J. Janssen, T. Reed & A. Wolf, 2008. Impacts of the introduced round goby (*Apollonia melanostoma*) on Dreissenids (*Dreissena polymorpha* and *bugensis*) and on macroinvertebrate community between 2003 and 2006 in the littoral zone of Green Bay, Lake Michigan. *Journal of Great Lakes Research* 34: 690–697.

- Leppäkoski, E. & S. Olenin, 2000. Non-native species and rates of spread: lessons from the brackish Baltic Sea. *Biological Invasions* 2: 151–163.
- MacArthur, R. H. & E. R. Pianka, 1966. On optimal use of a patchy environment. *American Naturalist* 100: 603–609.
- Manly, B. F. J., 2006. On a proposed method for analysing experiments on food choice. *Journal of Experimental Marine Biology and Ecology* 335: 154–155.
- Madenjian, C. P., M. A. Stapanian, L. D. Witzel, D. W. Einhouse, S. A. Pothoven & H. L. Whitford, 2011. Evidence for predatory control of the invasive round goby. *Biological Invasions* 13: 987–1002.
- Mansour, R. A. & R. N. Lipcius, 1991. Density-dependent foraging and mutual interference in blue crabs preying upon infaunal clams. *Marine Ecology Progress Series* 72: 239–246.
- Marentette, J. R. & S. Balshine, 2012. Altered prey responses in round goby from contaminated sites. *Ethology* 118: 1–9.
- Martin, G., J. Kotta, T. Möller & K. Herkül, 2013. Spatial distribution of marine benthic habitats in the Estonian coastal sea, northeastern Baltic Sea. *Estonian Journal of Ecology* 62: 165–191.
- Norling, P. & N. Kautsky, 2008. Patches of the mussel *Mytilus* sp. are islands of high biodiversity in subtidal sediment habitats in the Baltic Sea. *Aquatic Biology* 4: 75–87.
- Ojaveer, H., A. Jaanus, B. R. Mackenzie, G. Martin, S. Olenin, T. Radziejewska, I. Telesh, M. L. Zettler & A. Zaiko, 2010. Status of biodiversity in the Baltic Sea. *PLoS One* 5: e12467.
- Ojaveer, H. & J. Kotta, 2015. Ecosystem impacts of the widespread non-indigenous species in the Baltic Sea: literature survey evidences major limitations in knowledge. *Hydrobiologia* 750: 171–185.
- Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Polymos, M. H. H. Stevens, H. Wagner, 2015. Package 'vegan'. Community ecology package, version 2-2.
- Olenin, S. & E. Leppäkoski, 1999. Non-native animals in the Baltic Sea: alteration of benthic habitats in coastal inlets and lagoons. *Hydrobiologia* 393: 233–243.
- Post, J. R. & D. Cucin, 1984. Changes in the benthic community of a small precambrian lake following the introduction of yellow perch (*Perca flavescens*). *Canadian Journal of Fisheries and Aquatic Sciences* 41: 1496–1501.
- Raby, G. D., L. F. G. Gutowsky & M. G. Fox, 2010. Diet composition and consumption rate in round goby (*Neogobius melanostomus*) in its expansion phase in the Trent River, Ontario. *Environmental Biology of Fishes* 89: 143–150.
- Rakauskas, V., Ž. Pūtyš, J. Dainys, J. Lesutienė, L. Ložpys & K. Arbačiauskas, 2013. Increasing population of the invader round goby, *Neogobius melanostomus* (Actinopterygii: Perciformes: Gobiidae), and its trophic role in the Curonian Lagoon, SE Baltic Sea. *Acta Ichthyologica Et Piscatoria* 43: 95–108.
- Ray, W. J. & L. D. Corkum, 1997. Predation of zebra mussels by round gobies, *Neogobius melanostomus*. *Environmental Biology of Fishes* 50: 267–273.
- Ray, W. J. & L. D. Corkum, 2001. Habitat and site affinity of the round goby. *Journal of Great Lakes Research* 27: 329–334.
- Ribeiro, F., R. L. Orjuela, M. F. Magalhães & M. J. Collares-Pereira, 2007. Variability in feeding ecology of a South American cichlid: a reason for successful invasion in mediterranean-type rivers? *Ecology of Freshwater Fish* 16: 559–569.
- Sapota, M. R., 2004. The round goby (*Neogobius melanostomus*) in the Gulf of Gdansk – a species introduction into the Baltic Sea. *Hydrobiologia* 514: 219–224.
- Schreiber, S. J., 1997. Generalist and specialist predators that mediate permanence in ecological communities. *Journal of Mathematical Biology* 36: 133–148.
- Skazhkina, E. P. & V. A. Kostyuchenko, 1968. Food of *N. melanostomus* in the Azov Sea. *Voprosy Ikhtologiiy* 8: 303–311. (in Russian).
- Skora, K. E. & J. Rzeznik, 2001. Observations on diet composition of *Neogobius melanostomus* Pallas 1811 (Gobiidae, Pisces) in the Gulf of Gdansk (Baltic Sea). *Journal of Great Lakes Research* 27: 290–299.
- Sokołowska, E. & D. P. Fey, 2011. Age and growth of the round goby *Neogobius melanostomus* in the Gulf of Gdańsk several years after invasion. Is the Baltic Sea a new promised land? *Journal of Fish Biology* 78: 1993–2009.
- Strayer, D. L., V. T. Eviner, J. M. Jeschke & M. L. Pace, 2006. Understanding the long-term effects of species invasions. *Trends in Ecology and Evolution* 21: 645–651.
- Taplin, R. H., 2007. Experimental design and analysis to investigate predator preferences for prey. *Journal of Experimental Marine Biology and Ecology* 344: 116–122.
- Underwood, A. J. & K. R. Clarke, 2005. Solving some statistical problems in analyses of experiments on choices of food and on associations with habitat. *Journal of Experimental Marine Biology and Ecology* 318: 227–237.
- Underwood, A. J. & K. R. Clarke, 2006. Response on a proposed method for analysing experiments on food choice. *Journal of Experimental Marine Biology and Ecology* 335: 151–153.
- Underwood, A. J. & K. R. Clarke, 2007. More response on a proposed method for analysing experiments on food choice. *Journal of Experimental Marine Biology and Ecology* 344: 113–115.
- Underwood, A. J., M. G. Chapman & T. P. Crowe, 2004. Identifying and understanding ecological preferences for habitat or prey. *Journal of Experimental Marine Biology and Ecology* 300: 161–187.
- Veber, T., J. Kotta, V. Lauringson & I. Kotta, 2009. Influence of the local abiotic environment, weather and regional nutrient loading on macrobenthic invertebrate feeding groups in a shallow brackish water ecosystem. *Oceanologia* 51: 541–559.
- Volterra, V., 1928. Variations and fluctuations of the number of individuals in animal species living together. *Journal du Conseil International pour l'Exploration de la Mer* 3: 3–51.
- Westerbom, M., M. Kilpi & O. Mustonen, 2002. Blue mussels, *Mytilus edulis*, at the edge of the range: population structure, growth and biomass along a salinity gradient in the north-eastern Baltic Sea. *Marine Biology* 140: 991–999.
- Zavaleta, E. S., R. J. Hobbs & H. A. Mooney, 2001. Viewing invasive species removal in a whole-ecosystem context. *Trends in Ecology and Evolution* 16: 454–459.



ORIGINAL ARTICLE

Predator and prey: the role of the round goby *Neogobius melanostomus* in the western Baltic

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ABSTRACT

Different studies on the position of the non-indigenous species *Neogobius melanostomus* within the coastal food web of the Pomeranian Bay (western Baltic) were performed, resulting in a quantitative and qualitative species list of prey organisms found in the stomachs of the invader and an estimation concerning the importance of round goby as prey for different resident predators. It seems that the colonization process is not fully completed yet, but the results reveal that the species is already established in the food web 16 years after the first observation within the study area. The results show that *N. melanostomus* feed upon a wide range of different resident organisms. While a direct predation effect on native fish species appears rather unlikely, indirect effects such as competition cannot yet be excluded. In addition, our results reveal an ontogenetic diet shift and that the round goby itself already serves as an important prey for piscivorous fish and seabirds. Finally, we formulate different hypotheses based on our results which will require further research.

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Introduction

Non-indigenous species often pose a severe threat to the native brackish water fauna in the Baltic Sea (Lepäkoski & Olenin 2000, 2001). One of the most prominent invasive species is the euryhaline round goby *Neogobius melanostomus* (Pallas, 1814). Native to the Caspian Sea, Black Sea and the adjacent Sea of Azov (Svetovidov 1964; Miller 1986), it has been introduced into various locations (Kornis et al. 2012). In 1990, the round goby was first recorded in the St Clair River in North America and has spread throughout the Great Lakes since then (Jude et al. 1992, 1995). In Central Europe the species was observed first in the Baltic Sea (Skóra & Stolarski 1993) in 1990 and later in several river and canal systems (Wiesner 2005; van Beek 2006; Borchering et al. 2011; Piria et al. 2011; Verreycken et al. 2011; Brunken et al. 2012; Hempel & Thiel 2013; Jacobs & Hoedemakers 2013; Schomaker & Wolter 2014). In the Baltic Sea, round gobies were first caught in the Gulf of Gdansk in 1990 (Skóra & Stolarski 1993, 1996) and had become one of the dominant species in the western part of the Gulf by 1999 (Sapota & Skóra 2005). The invasion expanded in a northeasterly direction towards the Curonian Lagoon (Rakauskas

et al. 2013), the Gulf of Riga and the Gulf of Finland (Ojaveer 2006), as well as in a westerly direction (Winkler et al. 2000; Winkler 2006; Czugała & Woźniczka 2010; Schomaker & Wolter 2014). While several studies about the invasive process in the Great Lakes were published within recent decades (e.g. Barton et al. 2005; Lederer et al. 2006; Raby et al. 2010; Kipp et al. 2012; Kipp & Ricciardi 2012), the invasive process in the Baltic has been treated as a 'Cinderella subject' and in comparison fewer studies were published (e.g. Janssen & Jude 2001; Barton et al. 2005; Lederer et al. 2006; Copp et al. 2008; Raby et al. 2010; Kipp et al. 2012; Kipp & Ricciardi 2012; Sapota et al. 2014; Kotta et al. 2016). This has led to an opportunity to investigate the invasive and colonization process of a new species. However, in some areas the invasion process is still ongoing and should be investigated. One of those regions exhibiting an ongoing invasion process is the western part of the Baltic, where our studies were performed. The first occurrence of *N. melanostomus* in our study area was observed in 1999. In recent years, the species spread out rapidly and abundances increased (Winkler et al. 2015). Presuming that the species must already be established in the local food web to achieve the observed

successful colonization of the area, we present a synergistic compilation of several case studies – each focusing on a particular aspect of the trophodynamic interactions between the round goby and the food web in the Pomeranian Bay and adjacent waters. Combining different adapted techniques, we focused on important native components of the investigated ecosystem and examined their specific interaction with *N. melanostomus*.

Materials and methods

Top-down effect

To investigate the prey of *Neogobius melanostomus*, two different study sites in the Pomeranian Bay and adjacent waters were investigated (Figure 1, Case Study 1) including a semi-enclosed inshore lagoon (Greifswald Bay) and an area close to the Oderbank Plateau. At the first site (inshore), round goby samples were taken in three different habitat types using a beam trawl in August, October and November 2014. Habitat types included the ‘*Potamogeton* zone’ between 1 and 2 m water depth, the ‘*Zostera* zone’ at 3–4 m depth and the ‘Subphytial zone’ between 5 and 7 m water depth. Species

from Oderbank Plateau were sampled with multi-mesh gillnets at around 5, 10 and 20 m depths in May and November 2014 (Figure 1, Case Study 1).

Another round goby sampling was performed from May 2011 until July 2012 at the inshore lagoon (‘*Potamogeton* zone’) and the area of the Oderbank Plateau at depths up to 14 m with a beach seine and trawl, respectively (Figure 1, Case Study 2). In the laboratory, round gobies for both studies were measured for total length and stomach contents were examined. Gobies were dissected ventrally and the stomachs separated from the remaining digestive tract. Only prey organisms that had been in the stomach were identified to the lowest possible taxonomic level. For samples from 2014 the presence/absence of the single prey taxa was noted for each fish dissected and afterwards the percentage of specific prey taxa was calculated for each individual goby. For samples from 2011 and 2012, the frequency of occurrence of prey taxa was noted per length class.

Bottom-up effect

To examine the bottom-up effect of the invasive *Neogobius melanostomus*, stomach content analyses of piscivorous fish and analyses of pellets of the cormorant

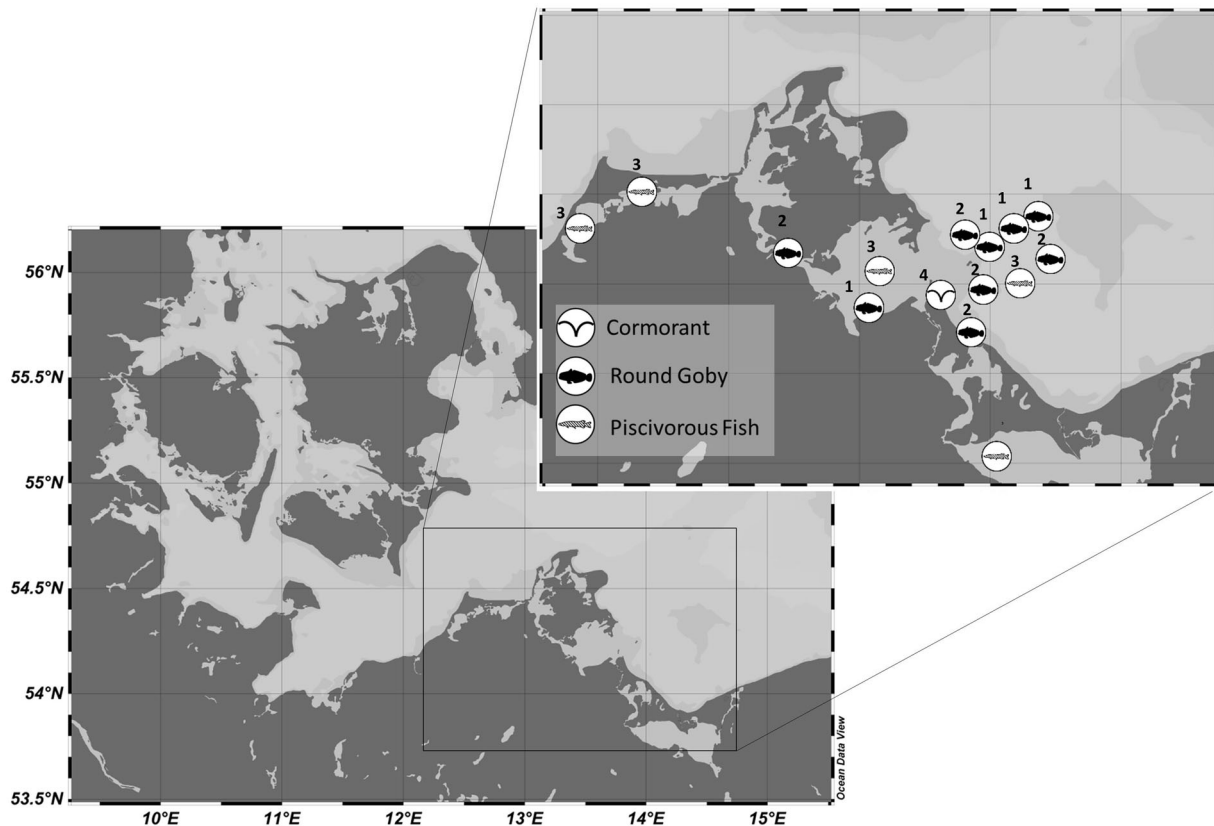


Figure 1. Sampling stations of cormorant pellets, round gobies and piscivorous fish in the Pomeranian Bay. Numbers indicate the case study number.

Phalacrocorax carbo (Linnaeus, 1758) from the Pomeranian Bay were investigated. The fish were sampled by different techniques (bottom trawl, gillnet) at several locations during the year where an occurrence of round gobies was noted (Figure 1, Case Study 3). Total length of piscivorous fish was measured to the nearest 0.5 cm, individuals were dissected ventrally and the stomach was separated. Afterwards, stomach contents were identified to the lowest possible taxon. When prey individuals were intact, the wet weight and length were noted; otherwise, individual lengths and weights were estimated using empirically characterized relations between total length/biomass and otoliths or other skeleton fragments from the literature and/or the local reference collection (Härkönen 1986, Debus & Winkler 1996; Leopold et al. 2001; Myts 2012). The diet composition was calculated as N_i (%) individual number, W_i (%) as the reconstructed weight and F_i (%) as the frequency of occurrence of prey taxon i . These three parameters were combined to calculate the Index of Relative Importance (IRI) as used by George & Hadley (1979):

$$\text{IRI} = \frac{N_i + W_i + F_i}{\sum (N_i + W_i + F_i)} \times 100\%$$

For the prey analysis of piscivorous birds, cormorant pellets were sampled between 2010 and 2015. The pellets were collected during the breeding season (between March and October) near Peenemünde in the Pomeranian Bay (Figure 1, Case Study 4). When possible, 30 or more pellets were collected at least once a month with fresh and intact pellets being preferred to older and/or damaged pellets. After sampling, pellets were washed so that macerated pellet contents and prey items could be identified more efficiently. All prey items were identified to the lowest possible taxon. At least, length and biomass of prey individuals were recalculated via published and non-published regressions based on the identified otoliths and bony prey remains. Regressions for otoliths and lengths and weights for the round goby were calculated according to Menge (2012).

Results

Top-down effect

Round gobies were found at each sample site except at a depth of 20 m on the Oderbank Plateau. The length and weight of each round goby was measured and individuals were assigned to different length classes (\leq 50 mm, 51–100 mm, 101–150 mm, 151–200 mm and 201–250 mm). When available, a maximum of 10

stomachs per length class and haul were analysed from the beam trawls and 20 stomachs per length class and depth contour were examined from the gillnet survey. A total of 1192 individuals were caught with 249 stomachs being analysed in the first case study, of which 47 were empty (Table I). Individuals at both study sites from Case Study 1 consumed a variety of prey organisms. In general, polychaetes belonging to the family Nereididae Blainville, 1818 were identified in stomachs. Arthropod prey items included insects and crustaceans, which comprised several taxonomic groups as well. *Neogobius melanostomus* fed on cladocerans (*Bosmina*), ostracods and copepods, but also on amphipods such as Gammaridae, isopods as *Idotea chelipes* (Pallas, 1766), decapods including *Crangon crangon* (Linnaeus, 1758) and *Palaemon* sp., and Balanidae. The round goby diet also included bivalves such as *Mya arenaria* Linnaeus, 1758, *Cerastoderma* spp., *Limecola balthica* (Linnaeus, 1758) and *Mytilus* sp., whereas gastropods included hydrobiids *Peringia ulvae* (Pennant, 1777) and/or *Ecrobia ventrosa* (Montagu, 1803) and *Littorina* spp. On the Oderbank Plateau round gobies also consumed *Hali-cryptus spinulosus* (von Siebold, 1849), which belong to the family Priapulidae. In the inshore lagoon (Greifswald Bay), the diet of smaller individuals ($<$ 50 mm TL) predominantly included ostracods, copepods and cladocerans. Larger round gobies ($>$ 100 mm TL) increasingly consumed polychaetes and molluscs. *Neogobius melanostomus* from the Pomeranian Bay (Oderbank Plateau), with a mean total length of 111.3 ± 13.5 mm for females and 150.5 ± 31.7 mm for males, fed on crustaceans such as *C. crangon* and *Palaemon* sp. However, molluscs were consumed more often than all other prey items such as arthropods, annelids and priapulids. *Mytilus* sp. and hydrobiid gastropods were identified as the most important prey items found in the stomachs of fish from the Oderbank Plateau. From the sampling in 2011 and 2012 (Case Study 2), a total of 115 round gobies were randomly selected depending on their length class so that around 20 stomachs per length class, if available, were analysed, resulting in 17 empty and 98 full or partly full stomachs. In addition to the above results, the analyses of prey occurrence of distinct length classes showed an ontogenetic diet shift. Crustaceans dominated the stomach contents of smaller individuals, while the occurrence of molluscs increased with body length (Figure 2).

Bottom-up effect

In addition to the stomach analysis of round gobies, a total of 321 individuals of piscivorous fish were analysed

Table 1. Detailed information about investigated round gobies (RG) and respective stomach contents from Case Study 1.

Location	Littoral zone									Oderbank Plateau			
	August		October			November			May		November		
	~1.5	~2.5	~1.5	~3.5	~6	~1.5	~3.5	~6	~5	~10	~5	~10	
RG mean total length (mm)	43.4 (±22.9)	34.6 (±11.1)	39.0 (±13.0)	27.5 (±4.1)	24.3 (±5.4)	37.4 (±6.7)	37.0 (±7.0)	29.3 (±13.2)	128.77 (±32.99)	123.95 (±32.36)	148.73 (±24.49)	136.92 (±25.27)	
Total number of RG	156	89	89	10	25	266	3	4	503	19	15	13	
Investigated stomachs	19	26	34	9	11	31	3	4	65	19	15	13	
Empty stomachs	7	4	3	1	3	8	0	0	13	4	4	0	
Prey presence %													
Polychaeta	26	19	15	0	9	35	0	25	21	7	0	15	
Nereididae	11	4	3	0	0	32	0	25	13	0	0	15	
Arthropoda	95	62	88	100	100	84	100	75	44	47	64	38	
Amphipoda	26	12	3	0	0	19	0	0	0	0	0	0	
<i>Corophium</i> sp.	0	4	0	0	0	6	0	0	0	0	0	0	
Gammaridae	5	0	3	0	0	10	0	0	0	0	0	0	
<i>Bosmina</i> sp.	0	0	9	0	0	45	0	0	0	0	0	0	
Copepoda	21	4	59	33	0	77	67	75	0	0	0	0	
Balanidae	0	0	0	0	0	0	0	0	23	20	55	31	
<i>Crangon crangon</i>	0	0	0	0	0	0	0	0	0	0	9	15	
<i>Palaemon</i> sp.									0	0	0	15	
Insecta	21	4	15	0	0	6	33	0	0	0	0	0	
Chironomidae	16	0	15	0	0	6	33	0	0	0	0	0	
Isopoda	32	8	21	0	0	0	0	0	0	0	0	0	
<i>Cyathura carinata</i>	0	0	3	0	0	0	0	0	0	0	0	0	
<i>Idotea chelipes</i>	32	0	18	0	0	0	0	0	0	0	0	0	
Ostracoda	11	12	44	100	100	29	67	25	0	0	0	0	
Mollusca	37	69	21	56	82	0	33	25	98	93	100	100	
Bivalvia	26	46	9	0	55	0	33	25	85	80	45	62	
<i>Cerastoderma</i> sp.	0	4	0	0	45	0	0	0	27	27	0	31	
<i>Limecola balthica</i>	5	0	3	0	9	0	0	0	4	7	0	0	
<i>Mya arenaria</i>	11	4	3	0	9	0	0	0	27	0	27	15	
<i>Mytilus</i> sp.	11	4	3	0	0	0	0	0	67	73	36	31	
Gastropoda	11	35	15	56	64	0	0	0	92	67	82	92	
Hydrobiid gastropods	11	31	6	0	0	0	0	0	92	67	82	92	
<i>Littorina</i> sp.	0	0	6	44	64	0	0	0					
Priapulidae	0	0	0	0	0	0	0	0	12	7	0	0	
<i>Halicryptus spinulosus</i>	0	0	0	0	0	0	0	0	12	7	0	0	

Prey presence is the percentage of the taxa (per line and column) occurring in non-empty stomachs based on presence/absence analysis.

Table II. Information about analysed piscivorous fish species (Case Study 3).

Species	Geographical area	Number	Total number	Non-empty stomachs
<i>Scophthalmus maximus</i> (Linnaeus, 1758)	Pomeranian Bay	8	8	8
<i>Perca fluviatilis</i> Linnaeus, 1758	Pomeranian Bay	91	186	104
	Greifswald Bay	19		
	Saaler Bodden & Darss-Zingst Lagoon	44		
	Odra estuary	32		
<i>Sander lucioperca</i> (Linnaeus, 1758)	Greifswald Bay	28	89	57
	Pomeranian Bay	29		
	Odra estuary	32		
<i>Gymnocephalus cernuus</i> (Linnaeus, 1758)	Greifswald Bay	3	38	12
	Pomeranian Bay	35		

(Table II, Case Study 3). Stomachs from *Scophthalmus maximus* (Linnaeus, 1758) and *Gymnocephalus cernuus* (Linnaeus, 1758) contained prey items, but no *Neogobius melanostomus* were detected. However, stomachs from *Perca fluviatilis* Linnaeus, 1758 and *Sander lucioperca* (Linnaeus, 1758) included *N. melanostomus* besides other prey species. The IRI shows that *N. melanostomus* became an important prey item for *S. lucioperca* and *P. fluviatilis* during the last years of the invasion process (Figure 3). These results were verified by determining the number, the frequency of occurrence and the biomass of the food items. Furthermore, *N. melanostomus* was found in the stomachs of *S. lucioperca* and *P. fluviatilis* at all sampling sites. Whereas *S. lucioperca* caught in the Odra estuary had the highest number of *N. melanostomus* in their stomachs, the IRI of *N. melanostomus* calculated for *P. fluviatilis* was highest from this location. In this study, *S. lucioperca* with a total length of 30–38 cm and *P. fluviatilis* with a total length of 20–30 cm had ingested the highest number of *N. melanostomus*.

Moreover, the analysis of 1048 (103–263 per year) cormorant pellets (Case Study 4) shows that the

occurrence of *N. melanostomus* in the pellets has increased significantly over recent years (Pearson's r , $p=0.009$). In 2010, the first round gobies were observed in the pellets; however, the percentage of

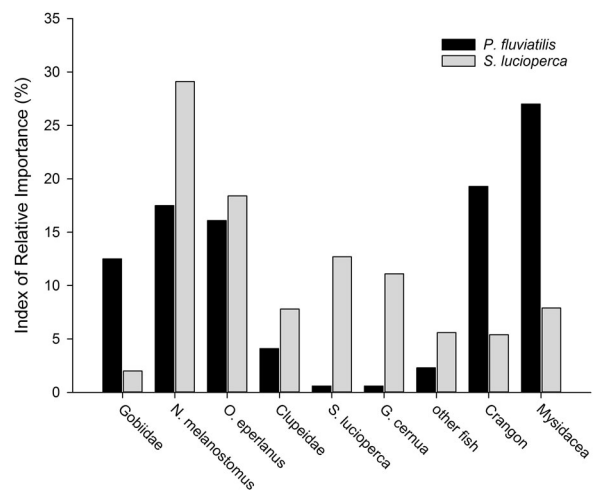


Figure 3. Diet composition of *Perca fluviatilis* in black ($n = 104$) and *Sander lucioperca* in grey ($n = 57$) expressed as Index of Relative Importance.

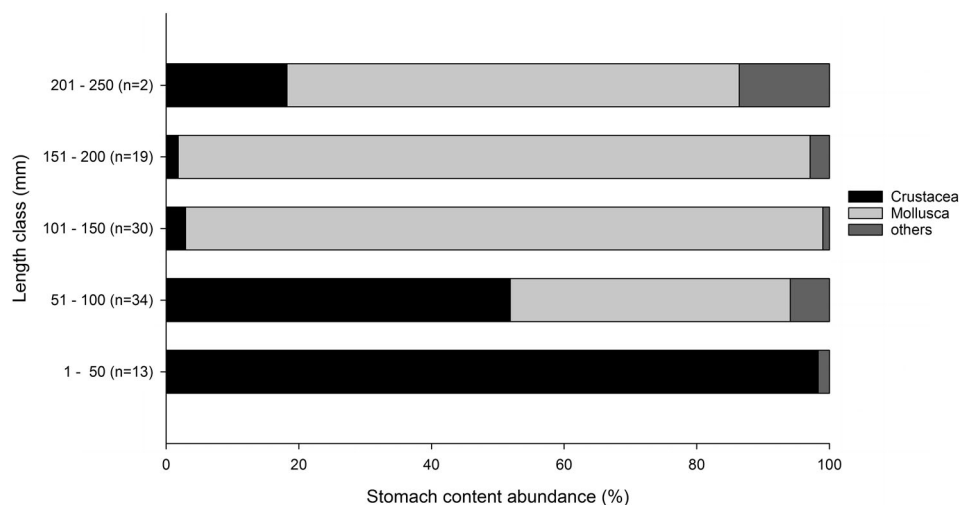


Figure 2. Frequency of occurrence of different prey taxa for different length classes, Case Study 2. Numbers in brackets indicate the number of non-empty analysed round goby stomachs.

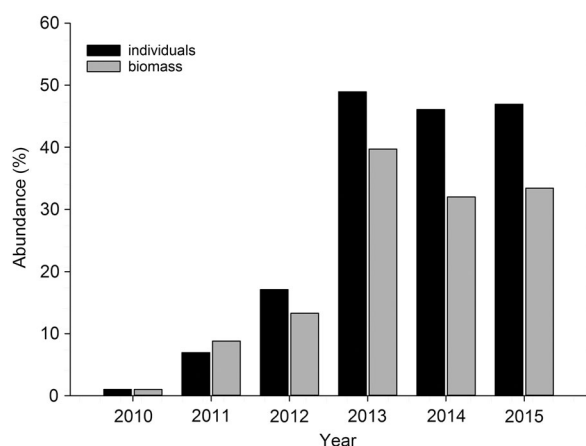


Figure 4. Number of individuals and estimated biomass of round goby as percentage of the total number and biomass of the cormorant pellets for 2010–2015 in Peenemünde (Case Study 4) (Stark 2011; Myts 2012; Winkler et al. 2014). Number of analysed cormorant pellets: 2010 = 263, 2011 = 221, 2012 = 184, 2013 = 164, 2014 = 103, 2015 = 113.

biomass was very small. Over the following three years, the occurrence of *N. melanostomus* within the pellets increased considerably, especially between 2012 and 2013 (Figure 4). In recent years, the biomass of *N. melanostomus* represented about 35% of the estimated pellet biomass. This shows that *N. melanostomus* became an important, perhaps the most important, prey of the cormorants at that location during the breeding season. In contrast, the biomass of other prey organisms decreased over the last six years.

With an occurrence of 20%, for example, roach *Rutilus rutilus* (Linnaeus, 1758) was the most important prey in 2010, while the percentage was only around 4% in 2015 (Figure 5).

Discussion

Despite the emergence of modern techniques such as measuring stable isotopes and fatty acid analyses, classical stomach content analysis remains an irreplaceable method to investigate fish feeding ecology, mainly due to the advantage that prey items can be determined to lower taxonomic levels (Cresson et al. 2014). However, it has to be considered that prey items are sometimes very difficult to identify, depending on their degree of digestion (Hyslop 1980), and that some species could be overestimated due to indigestible fractions (Hyslop 1980; Baker et al. 2014). In our study, the percentage of bivalves might be overvalued in the diet of round goby because their shells are indigestible and have a longer retention time in stomachs compared to soft-bodied prey. Moreover, they are easier to identify (Coulter et al. 2011; Brush et al. 2012). Another critical aspect is that the digestive process continues during the catch period (Baker et al. 2014); therefore, fish were frozen immediately after capture and the fishing time of the gillnets was minimized to less than 24 hours. However, even if the stomach content analyses present only a snapshot of the species' diet and describe what the individual has consumed over a certain time span, the results of this study

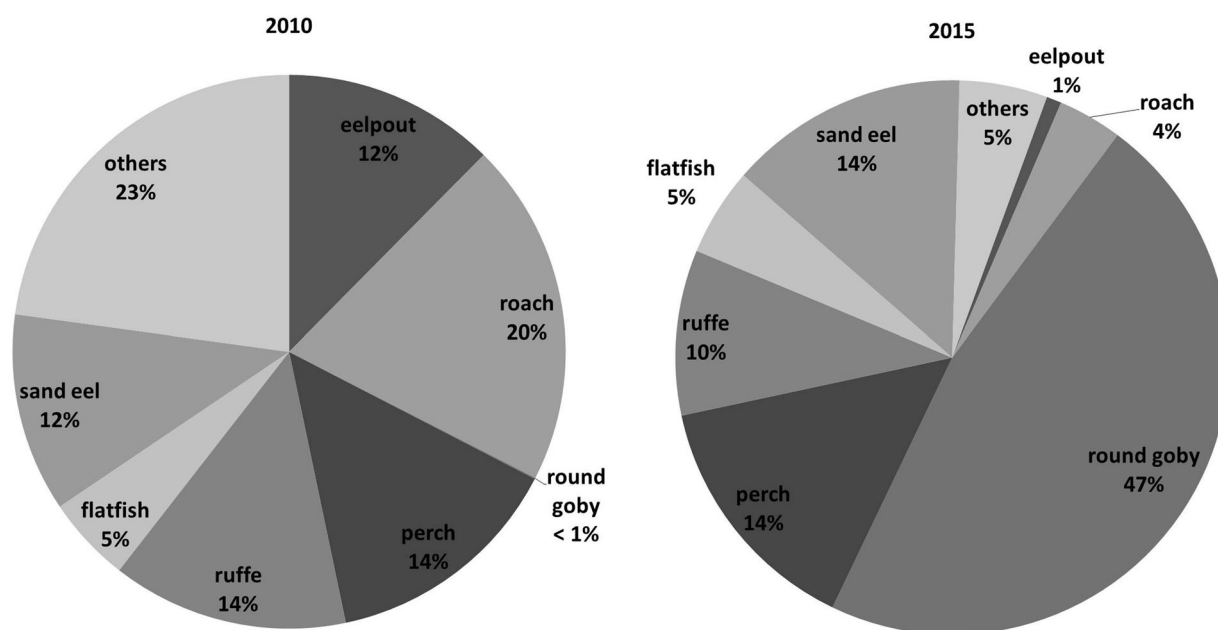


Figure 5. Abundance of fish species in cormorant pellets from 2010 (first appearance of round goby in prey) compared to 2015 (Case Study 4). Data for 2010 modified from Stark (2011). Number of analysed cormorant pellets: 2010 = 263, 2015 = 113.

regarding dietary composition of the round goby and round goby predators are assumed to represent a reasonably accurate picture within the different case studies.

Our results show that the round goby preys upon a variety of resident species and shifts the choice of its prey depending on its size. The study reveals that mainly crustaceans and molluscs were impacted directly by the introduction of round goby while fish were rarely found in the stomachs. Therefore, a significant and direct impact on fish species appears rather unlikely while an indirect impact on the resident fish community due to competition could not be excluded at this stage and will be discussed later. On the other hand, some piscivorous fish and bird species adapted to the new food resource so that *Neogobius melanostomus* became an important food item.

The percentage of successful invaders is difficult to determine. Statistics are rare and unsuccessful Non-Indigenous Species (NIS) are underrepresented, as well as the more easily observable species being overrepresented (Lodge 1993). A review conducted by Lodge (1993) presents a minimum rate of NIS establishment of 35%, but others assume a rate of 10% (Williamson & Brown 1986; Williamson 1989). The colonization success of NIS depends on different factors, but it is assumed that climate and predation are the most important factors influencing the invasion process; however, competition, diseases and other factors are difficult to analyse and could therefore be undervalued (Crawley et al. 1986; Lodge 1993). On the other hand, the most important impact of introduced mammals on native species, for example, is caused by predation and habitat changes. Ecological changes due to competition are less frequently documented, probably due to the challenging task of proving their actual significance (Ebenhard 1988). Based on different types of fishing gear (e.g. bottom trawls, beach seine) and interviews with fishermen, a chronological analysis of the round goby invasion in the eastern German part of the Baltic shows that *N. melanostomus* has occurred in the German part of the Pomeranian Bay since 1999 and spread out rapidly over recent years with an increasing abundance at some sites (Winkler 2006; Winkler et al. 2015). Such a fast and successful colonization can only be feasible with a successful niche partitioning together with the factors described above. In addition, our case studies reveal that *N. melanostomus* is already successfully established in the food web of our study area and has already or is well on the way to establishing itself within the ecosystem in the investigated area. Therefore, future work should focus on the description of the niche, and the

direct and indirect influence on native species and habitats. As an example, apart from *N. melanostomus*, we identified 12 other fish species within the inshore and 19 other fish species within the offshore study area. While our results support the findings of Thiel et al. (2014) that it can be excluded that round gobies exert a high predation pressure on native fish species, it can be assumed that there is already a potential competition concerning space and food resources at least with some of the native species. As our results show, smaller *N. melanostomus* feed upon crustaceans while larger individuals prefer molluscs. A similar feeding behaviour and therefore a potential competition is assumed for *Platichthys flesus* (Linnaeus, 1758) and *Vimba vimba* (Linnaeus, 1758) in the Baltic Sea. Young of the year (YOY) flounders start feeding on small crustaceans, larvae of chironomids and oligochaetes, whereas they switch to polychaetes and molluscs at a body length of around 10 cm (Ojaveer & Drevs 2003). YOY vimba bream feed on small crustaceans, molluscs (*Hydrobia*) and mainly on the polychaete *Hediste diversicolor* (O.F. Müller, 1776) in the Pärnu Bay of the Baltic Sea. *Limecola balthica* is dominant in the diet of larger vimba bream (Erm et al. 2003). In addition, molluscs are also an important food for *Rutilus rutilus* (Vetemaa et al. 2003), whereby a competition with *N. melanostomus* may exist. Karlson et al. (2007) describe a dietary overlap between small flounders and round gobies based on stomach contents, stable isotope analyses and lab experiments and reveal a decrease of the flounder population following the establishment of round goby in the area. A comparison between stomach contents from 0 age flounders (Andersen et al. 2005) and our results, in addition to the known temporal and spatial overlap at our study sites, supports the potential diet competition. However, besides the indirect diet competition with other fish species, direct feeding consequences exerted by *N. melanostomus* within the ecosystem are still unknown. As an example, our data show that isopods (*Idotea chelipes*) and hydrobiid gastropods were regularly found in stomach contents of round goby within the inshore area. Both prey species are grazers within the macrophyte area (Schaffelke et al. 1995; Schanz et al. 2000) and an induced decrease of these grazers due to *N. melanostomus* predation could have extensive consequences for the filamentous algae fouling on seagrass and other macrophytes and thus for the whole ecosystem. Besides the negative effects on the native biodiversity, our analyses of the cormorant pellets and stomach contents of piscivorous fishes show that the biomass of native species within the prey decreased, which may have positive effects

on the population of those resident species. For example, round goby is one of the most important food items for pikeperch in the Sea of Azov (Maiskij 1955). Therefore, it is not surprising that round goby is now the most important food item for pikeperch in the newly colonized Kiel Canal (Thiel et al. 2014). Gobies also serve as an important food especially for young sea mammals. According to Behnke et al. (1998), 50% of the food biomass of the harbour porpoises *Phocoena phocoena* (Linnaeus, 1758) in the Baltic Sea consists of gobies. Hepner et al. (1976) reviewed literature concerning the diet of harbour porpoises in the Black Sea and found that a total of 36% of the porpoises' food biomass consisted of gobies, including *N. melanostomus*. However, an assumption about future consequences would be purely speculative. Therefore, more fieldwork and experiments are necessary to rate the consequences of the invasion of round goby for native biodiversity in the coastal area in order to learn more about the impact of general invasion processes.

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Disclosure statement

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References

- Andersen BS, Carl JD, GrønkJær P, Støttrup JG. 2005. Feeding ecology and growth of age 0 year *Platichthys flesus* (L.) in a vegetated and a bare sand habitat in a nutrient rich fjord. *Journal of Fish Biology* 66:531–52. doi:10.1111/j.0022-1112.2005.00620.x
- Baker R, Buckland A, Sheaves M. 2014. Fish gut content analysis: robust measures of diet composition. *Fish and Fisheries* 15:170–77. doi:10.1111/faf.12026
- Barton DR, Johnson RA, Campbell L, Petruniak J, Patterson M. 2005. Effects of round gobies (*Neogobius melanostomus*) on dreissenid mussels and other invertebrates in eastern Lake Erie, 2002–2004. *Journal of Great Lakes Research* 31 (Suppl 2):252–61. doi:10.1016/S0380-1330(05)70318-X
- Behnke H, Siebert U, Lick R, Bandomir B, Weiss R. 1998. The current status of harbour porpoises (*Phocoena phocoena*) in German waters. *Archive of Fishery and Marine Research* 46(2):97–123.
- Borcherding J, Staas S, Krüger S, Ondračková M, Šlapanský L, Jurajda P. 2011. Non-native gobiid species in the lower River Rhine (Germany): recent range extensions and densities. *Journal of Applied Ichthyology* 27:153–55. doi:10.1111/j.1439-0426.2010.01662.x
- Brunken H, Castro J, Hein M, Verwold A, Winkler HM. 2012. First records of round goby *Neogobius melanostomus* (Pallas, 1814) in the river Weser. *Lauterbornia* 75:31–37.
- Brush JM, Fisk AT, Hussey NE, Johnson TB. 2012. Spatial and seasonal variability in the diet of round goby (*Neogobius melanostomus*): stable isotopes indicate that stomach contents overestimate the importance of dreissenids. *Canadian Journal of Fisheries and Aquatic Sciences* 69:573–86. doi:10.1139/f2012-001
- Copp G, Kováč V, Zweimüller I, Dias A, Nascimento M, Balážová M. 2008. Preliminary study of dietary interactions between invading Ponto-Caspian gobies and some native fish species in the River Danube near Bratislava (Slovakia). *Aquatic Invasions* 3:193–200. doi:10.3391/ai.2008.3.2.10
- Coulter DP, Murry BA, Webster WC, Uzarski DG. 2011. Effects of dreissenid mussels, chironomids, fishes, and zooplankton on growth of round goby in experimental aquaria. *Journal of Freshwater Ecology* 26:155–62. doi:10.1080/02705060.2011.553987
- Crawley MJ, Kornberg H, Lawton TH, Usher MB, Southwood R, O'Connor RJ, Gibbs A. 1986. The population biology of invaders: Discussion. *Philosophical Transactions of the Royal Society B* 314:711–31. doi:10.1098/rstb.1986.0082
- Cresson P, Ruitton S, Ourgaud M, Harmelin-Vivien M. 2014. Contrasting perception of fish trophic level from stomach content and stable isotope analyses: a Mediterranean artificial reef experience. *Journal of Experimental Marine Biology and Ecology* 452:54–62. doi:10.1016/j.jembe.2013.11.014
- Czugała A, Woźniczka A. 2010. The river Odra estuary – another Baltic Sea area colonized by the round goby *Neogobius melanostomus* Pallas, 1811. *Aquatic Invasions* 5(1):561–65. doi:10.3391/ai.2010.5.1.014
- Debus L, Winkler HM. 1996. Hinweise zur computergestützten Auswertung von Nahrungsanalysen. *Rostocker Meeresbiologische Beiträge* 4:97–110.
- Ebenhard T. 1988. Introduced birds and mammals and their ecological effects. *Swedish Wildlife Research* 13(4):1–107.
- Erm V, Turovski A, Paaver T. 2003. Vimba bream, *Vimba vimba* (L.). In: Ojaveer E, Pihu E, Saat T, editors. *Fishes of Estonia*. Tallin: Estonian Academy Publishers, p 223–25.
- George EL, Hadley WF. 1979. Food and habitat partitioning between rock bass (*Ambloplites rupestris*) and smallmouth bass (*Micropterus dolomieu*) young of the year. *Transactions of the American Fisheries Society* 108:253–61. doi:10.1577/1548-8659(1979)108<253:FAHPBR>2.0.CO;2
- Härkönen T. 1986. Guide to the Otoliths of the Bony Fishes of the Northeast Atlantic. Hellerup, Denmark: Danbiu Aps. 256 pages.
- Hempel M, Thiel R. 2013. First records of the round goby *Neogobius melanostomus* (Pallas, 1814) in the Elbe River,

- Germany. *BiolInvasions Records* 2:291–95. doi:10.3391/bir.2013.2.4.05
- Heptner WG, Tshapskii KK, Arsenev VA, Sokolov VE. 1976. *Mlekopitajushstshie Sovjetskogo Sojusa*. Volume 2, Part 3. Lastonogie i Zubatye Kity [Mammals of the Soviet Union, Pinnipeds and Toothed Whales]. Moscow: Vysshaja Skola, 718 pages. (in Russian).
- Hyslop EJ. 1980. Stomach contents analysis – a review of methods and their application. *Journal of Fish Biology* 17:411–29. doi:10.1111/j.1095-8649.1980.tb02775.x
- Jacobs P, Hoedemakers K. 2013. The round goby *Neogobius melanostomus* (Pallas, 1814) (Perciformes: Gobiidae), an invasive species in the Albert Canal (Belgium). *Belgian Journal of Zoology* 143:148–53.
- Janssen J, Jude DJ. 2001. Recruitment failure of mottled sculpin *Cottus bairdi* in Calumet Harbor, Southern Lake Michigan, induced by the newly introduced round goby *Neogobius melanostomus*. *Journal of Great Lakes Research* 27:319–28. doi:10.1016/S0380-1330(01)70647-8
- Jude DJ, Reider RH, Smith W. 1992. Establishment of Gobiidae in the Great Lakes Basin. *Canadian Journal of Fisheries and Aquatic Sciences* 49:416–21. doi:10.1139/f92-047
- Jude DJ, Janssen J, Crawford G. 1995. Ecology, distribution, and impact of the newly introduced round & tubenose gobies on the biota of the St. Clair & Detroit rivers. In: Munawar M, Edsall T, Leach J, editors. *The Lake Huron Ecosystem: Ecology, Fisheries and Management*. Amsterdam: SPB Academic Publishing, p 447–60.
- Karlson AML, Almqvist G, Skóra KE, Appelberg M. 2007. Indications of competition between non-indigenous round goby and native flounder in the Baltic Sea. *ICES Journal of Marine Science* 64:479–86. doi:10.1093/icesjms/fsl049
- Kipp R, Ricciardi A. 2012. Impacts of the Eurasian round goby (*Neogobius melanostomus*) on benthic communities in the upper St. Lawrence River. *Canadian Journal of Fisheries and Aquatic Sciences* 69:469–86. doi:10.1139/f2011-139
- Kipp R, Hébert I, Lacharité M, Ricciardi A. 2012. Impacts of predation by the Eurasian round goby (*Neogobius melanostomus*) on molluscs in the upper St. Lawrence River. *Journal of Great Lakes Research* 38:78–89. doi:10.1016/j.jglr.2011.11.012
- Kornis MS, Mercado-Silva N, vander Zanden MJ. 2012. Twenty years of invasion: a review of round goby *Neogobius melanostomus* biology, spread and ecological implications. *Journal of Fish Biology* 80:235–85. doi:10.1111/j.1095-8649.2011.03157.x
- Kotta J, Nurkse K, Puntila R, Ojaveer H. 2016. Shipping and natural environmental conditions determine the distribution of the invasive non-indigenous round goby *Neogobius melanostomus* in a regional sea. *Estuarine, Coastal and Shelf Science* 169:15–24. doi:10.1016/j.ecss.2015.11.029
- Lederer A, Massart J, Janssen J. 2006. Impact of round gobies (*Neogobius melanostomus*) on dreissenids (*Dreissena polymorpha* and *Dreissena bugensis*) and the associated macro-invertebrate community across an invasion front. *Journal of Great Lakes Research* 32:1–10. doi:10.3394/0380-1330(2006)32[1:IORGNM]2.0.CO;2
- Leopold MF, van Damme CJ, Philippart CJM, Winter CJN. 2001. Otoliths of North Sea Fish – Fish Identification Key by Means of Otoliths and Other Hard Parts. World Biodiversity Database CD-ROM series. Amsterdam: ETI Bioinformatics. CD-ROM.
- Leppäkoski E, Olenin S. 2000. Non-native species and rates of spread: lessons from the brackish Baltic Sea. *Biological Invasions* 2:151–63. doi:10.1023/A:1010052809567
- Leppäkoski E, Olenin S. 2001. The meltdown of biogeographical peculiarities of the Baltic Sea: the interaction of natural and man-made processes. *AMBIO: A Journal of the Human Environment* 30:202–09. doi:10.1579/0044-7447-30.4.202
- Lodge DM. 1993. Biological invasions: lessons for ecology. *Trends in Ecology & Evolution* 8(4):133–37. doi:10.1016/0169-5347(93)90025-K
- Maiskij VN. 1955. Pitanie i kormovaja baza sudaka v Azovskom More [Feeding and food base of pikeperch in the Azov Sea]. *Trudy Vsesojuznyj Naucno-Issledovatel'skij Institut Morskogo Rybnogo Chozjajstva i Okeanografii (VNIRO)* 31:337–55. (in Russian)
- Menge M. 2012. Untersuchungen zur Ökologie der Schwarzmundgrundel (*Neogobius melanostomus*) in Mecklenburg-Vorpommern. Diploma Thesis. University of Rostock. 101 pages.
- Miller P. 1986. Gobiidae. In: Whitehead PJP, Bauchot M-L, Hureau J-C, Nielsen J, Tortonese E, editors. *Fishes of the North-Eastern Atlantic and the Mediterranean*, Volume 3. Paris: Unesco, p 1019–85.
- Myts D. 2012. Nahrungsökologie des Kormorans (*Phalacrocorax carbo sinensis*). Diploma Thesis. University of Rostock. 78 pages.
- Ojaveer H. 2006. The round goby *Neogobius melanostomus* is colonising the NE Baltic Sea. *Aquatic Invasions* 1:44–45. doi:10.3391/ai.2006.1.1.11
- Ojaveer E, Drevs T. 2003. Flounder, *Platichthys flesus trachurus* (Duncker). In: Ojaveer E, Pihu E, Saat T, editors. *Fishes of Estonia*. Tallin: Estonian Academy Publishers, p 367–68.
- Piria M, Šprem N, Jakovlić I, Tomljanović T, Matulić D, Treer T, et al. 2011. First record of round goby, *Neogobius melanostomus* (Pallas, 1814) in the Sava River, Croatia. *Aquatic Invasions* 6(Suppl 1):153–57. doi:10.3391/ai.2011.6.S1.034
- Raby GD, Gutowsky LFG, Fox MG. 2010. Diet composition and consumption rate in round goby (*Neogobius melanostomus*) in its expansion phase in the Trent River, Ontario. *Environmental Biology of Fishes* 89:143–50. doi:10.1007/s10641-010-9705-y
- Rakauskas V, Pütys Ž, Dainys J, Lesutiene J, Ložys L & Arbačiauskas K. 2013. Increasing population of the invader round goby, *Neogobius melanostomus* (Actinopterygii: Perciformes: Gobiidae), and its trophic role in the Curonian Lagoon, SE Baltic Sea. *Acta Ichthyologica et Piscatoria* 43:95–108. doi:10.3750/AIP2013.43.2.02
- Sapota M, Skóra K. 2005. Spread of alien (non-indigenous) fish species *Neogobius melanostomus* in the Gulf of Gdańsk (south Baltic). *Biological Invasions* 7:157–64. doi:10.1007/s10530-004-9035-0
- Sapota MR, Balazy P, Mirny Z. 2014. Modification in the nest guarding strategy – one of the reasons of the round goby (*Neogobius melanostomus*) invasion success in the Gulf of Gdansk. *International Journal of Oceanography and Hydrobiology* 43:21–28.
- Schaffelke B, Evers D, Walhorn A. 1995. Selective grazing of the isopod *Idothea baltica* between *Fucus evanescens* and *F. vesiculosus* from Kiel Fjord (Western Baltic). *Marine Biology* 124:215–18. doi:10.1007/BF00347125

- Schanz A, Polte P, Asmus H, Asmus R. 2000. Currents and turbulence as a top-down regulator in intertidal seagrass communities. *Biologia Marina Mediterranea* 7:278–81.
- Schomaker C, Wolter C. 2014. First record of the round goby *Neogobius melanostomus* (Pallas, 1814) in the lower River Oder, Germany. *BiolInvasions Records* 3:185–88. doi:10.3391/bir.2014.3.3.08
- Skóra KE, Stolarski J. 1993. New fish species in the Gulf of Gdańsk, *Neogobius* sp. [cf. *Neogobius melanostomus* (Pallas 1811)]. *Bulletin of the Sea Fisheries Institute, Gdynia* 1:83.
- Skóra KE, Stolarski J. 1996. *Neogobius melanostomus* (Pallas 1811), a new immigrant species in the Baltic Sea. *Proceedings of the Second International Symposium held in Gdansk, 18–22 October 1993. Crangon No. 1, Issues of the Marine Biology Centre in Gdynia*, p 101–08.
- Stark K. 2011. Nahrungsanalyse beim Kormoran (*Phalacrocorax carbo*). Diploma Thesis. University of Rostock. 106 pages.
- Svetovidov AN. 1964. Ryby Tshernogo Morja [Fishes of the Black Sea]. Moscow, Leningrad: ANSSSR izd. Nauka, p 435–40. (in Russian)
- Thiel R, Horn L, Knörr C, Tonn M. 2014. Analyse der ökologischen Einnischung der invasiven Schwarzmundgrundel in relevanten Brack und Süßgewässerhabitaten Schleswig Holsteins. Final Report. Landesamt für Landwirtschaft, Umwelt und ländliche Räume Schleswig Holstein. 199 pages.
- van Beek G. 2006. The round goby *Neogobius melanostomus* first recorded in the Netherlands. *Aquatic Invasions* 1:42–43. doi:10.3391/ai.2006.1.1.10
- Verreycken H, Breine JJ, Snoeks J, Belpaire C. 2011. First record of the round goby, *Neogobius melanostomus* (Actinopterygii: Perciformes: Gobiidae) in Belgium. *Acta Ichthyologica et Piscatoria* 41:137–40. doi:10.3750/AIP2011.41.2.11
- Vetemaa M, Saat T, Paaver T, Turovski A. 2003. Roach, *Rutilus rutilus* (L.). In: Ojaveer E, Pihu E, Saat T, editors. *Fishes of Estonia*. Tallin: Estonian Academy Publishers, p 167–68.
- Wiesner C. 2005. New records of non-indigenous gobies (*Neogobius* spp.) in the Austrian Danube. *Journal of Applied Ichthyology* 21:324–27. doi:10.1111/j.1439-0426.2005.00681.x
- Williamson M. 1989. Mathematical models of invasion. In: Drake JA, Mooney HA, di Castri F, Groves RH, Kruger FJ, Rejmanek M, Williamson M, editors. *Biological Invasions: A Global Perspective*. London: John Wiley and Sons, p 329–50.
- Williamson MH, Brown KC, Holdgate MW, Kornberg H, Southwood R, Mollison D. 1986. The analysis and modelling of British invasions (and discussion). *Philosophical Transactions of the Royal Society B* 314:505–22. doi:10.1098/rstb.1986.0070
- Winkler HM. 2006. Die Fischfauna der südlichen Ostsee, Bemerkungen zum gegenwärtigen Kenntnisstand. *Meeresangler-Magazin* 16:17–18.
- Winkler HM, Skora K, Repecka R, Pliks M, Neelov A, Urho L, et al. 2000. Checklist and status of fish species in the Baltic Sea. CM 2000/Mini: 11, ICES Paper. 6 + 9 pages.
- Winkler HM, Myts D, Lüttkemöller E, Gröger J. 2014. Ernährung des Kormorans und sein Einfluss auf die Fischbestände der Küstengewässer Vorpommerns. In: *Populationsanalyse und Erprobung von Maßnahmen zur Reduzierung des Bruterfolges beim Kormoran (Phalacrocorax carbo sinensis) in M-V sowie Untersuchungen über seinen Einfluss auf freilebende Fischbestände*. Landesförderinstitut Mecklenburg-Vorpommern, Abteilung Agrar-, Forst- u. Fischereiförderung.
- Winkler H, Kotterba P, Oesterwind D. 2015. Round goby: a story of invasion success in the Baltic. Poster in: ICES Annual Science Conference 2015, 21–25 September, Copenhagen, Denmark.

Feeding ecology of the invasive round goby
(*Neogobius melanostomus*) in contrasting
environments

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Feeding activity and diet composition of round goby (*Neogobius melanostomus*, Pallas 1814) in the coastal waters of SE Baltic Sea

by

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Abstract

Feeding activity and diet composition of round goby were investigated in the south-eastern Baltic Sea, the Lithuanian coastal waters during May-October 2012 in order to determine main feeding objects and seasonal periods when native fauna could be most affected by predation of this highly invasive species. In total, prey represented by 18 taxa was found in the gut contents of dissected fish. Feeding activity of round goby varied depending on the body size, sex and stage of the reproduction period. The gut contents of <50 mm specimens were dominated by zooplanktonic and meiobenthic organisms, whereas larger individuals (50-99 mm) shifted to amphipods and mollusks. Individuals of the intermediate 100-200 mm length had a variable diet, changing depending on the season; in spring they mostly preyed on *Macoma balthica*, in summer – on polychaetes, while in autumn the contribution of *Mytilus trossulus* and fish considerably increased in their diet. Diet composition of individuals ≥200 mm was relatively constant in the course of the study with substantial preference to *M. balthica*. These findings imply that benthic fauna, particularly a newly settled generation of epibenthic mollusks in autumn is under strong predatory pressure of the round goby.

Key words: invasive species, food composition, seasonal variability, reproductive cycle

Introduction

The round goby (*Neogobius melanostomus*, Pallas 1814) is a small bottom-dwelling fish species, originating from the Ponto-Caspian region: the Black Sea, the Caspian Sea, the Sea of Azov and the Sea of Marmara. During the last 15 years, this fish colonized Laurentian Great Lakes (Jude et al. 1992), some of their tributaries (Phillips et al. 2003; Krakowiak & Pennuto 2008; Pennuto et al. 2010), the Baltic Sea (Corkum et al. 2004) and many Eurasian rivers (Copp et al. 2005). In the Baltic Sea, round gobies were first reported in 1990 from the Gulf of Gdańsk, near the harbors of Hel and Gdynia. Several years later their population significantly increased in numbers and began to spread to the other parts of the Gulf of Gdańsk, mouth of the Vistula River, adjacent canals, the Vistula Lagoon (Sapota & Skóra 2005) and successfully expanded to the south-eastern and northern Baltic coastal areas (Sapota 2004; Ojaveer 2006; Björklund & Almqvist 2010). The first specimen of round goby in the Lithuanian coastal waters of the Baltic Sea was captured in the vicinity of Klaipėda harbor in 2002 (Zolubas 2003). Within a decade from initial introduction, abundance of round goby increased dramatically, allowing this fish to become a key component of ichthyofauna in the Lithuanian aquatory, including adjacent waters of the Curonian Lagoon (Rakauskas et al. 2013).

Expanding population of round goby has a great potential to change invaded ecosystem reducing diversity and abundance of its feeding objects (Barton et al. 2005; Krakowiak & Pennuto 2008), competing for various resources with native demersal fish species (Balshine et al. 2005; Karlson et al. 2007) or becoming an important component in the diet of piscivorous fish (Almqvist et al. 2010; Płachocki et al. 2012), birds (Bzoma 1998; Pūtys & Zarankaitė 2010) and mammals (Lundström et al. 2010). Round goby is a typical benthophagous fish, feeding predominantly on mollusks at the size of >100 mm (Skóra & Rzeznik 2001; Brush et al. 2012), while individuals <100 mm consume mainly zooplankton and other soft-bodied prey (Walsh et al. 2007; Hayden & Miner 2009; Thompson & Simon 2014). Feeding habits of this species are opportunistic and flexible, reflected in highly variable prey composition, depending on the body size, the type of

inhabited waterbody, biotope, diurnal and seasonal changes in the environment (Kornis et al. 2012).

Recently many researches on feeding ecology of round goby in European riverine (Kakareko et al. 2005; Borza et al. 2009; Polačik et al. 2009; Borcherding et al. 2013; Brandner et al. 2013; Vašek et al. 2014), estuarine (Rakauskas et al. 2008, 2013) and marine (Skóra & Rzeznik 2001; Wandzel 2003) ecosystems have been conducted. Only several of them, performed in lotic waterbodies, were focused on seasonal changes in feeding activity and diet composition (Borcherding et al. 2013; Vašek et al. 2014). No comprehensive research on the variation of seasonal feeding patterns in relation to the reproductive cycle has been performed in the Baltic Sea yet, which makes accurate evaluation of round goby impact on native zoobenthic communities difficult.

The goal of this study was to examine feeding patterns of round goby in the exposed coastal soft bottom area of SE Baltic Sea with an adjacent fragment of artificial hard substrate. The main objectives were:

- (1) to determine changes in the feeding activity of female and male round gobies during different months and stages of the reproduction period;
- (2) to evaluate seasonal and ontogenetic variation in the diet of differently sized individuals applying gut content analysis.

Materials and methods

Study site and sampling

This study was conducted in the south-eastern Baltic Sea, the Lithuanian coastal waters, close to the Klaipėda strait and harbor (56°43'34"N, 21°04'37"E). Seabed at the sampling site is dominated by soft and sandy sediments, strongly affected by wave action (Olenin & Daunys 2004), however, underwater harbor constructions and breakwater provide shelter and hard substrate for fish and invertebrates. Specific and variable environmental conditions determine high diversity and abundance of potential feeding objects for round gobies: soft sediments are inhabited by burrowing infaunal and actively swimming nectobenthic organisms (Olenin & Daunys 2004), whereas hard artificial substrates are colonized by

sessile epifauna and seasonal algae (Olenin et al. 1996), used as refugia by mobile macroinvertebrates and fish fry.

Sampling was performed at 2-3 week intervals in May-October 2012, while in the other months, round gobies were absent at the study site. Fish were collected using multi mesh gill nets (mesh size = 17.5, 20, 38 mm) at depths ranging from 6 to 11 m within 12 h. Small round gobies usually inhabit shallower waters compared to large specimens (Kornis et al. 2012) and cannot be caught with gill nets, therefore baited minnow trap (70 cm length, 25 cm height and 2.5 mm mesh size) was placed at a depth of 2-3 m to collect <100 mm specimens. Dead Baltic herrings (*Clupea harengus membras*) were used as a bait. Trapping was possible only during low waving and was conducted daily under suitable environmental conditions by checking the trap, taking out all captured animals and changing the bait. Individuals <100 mm did not occur in the trap until late summer and were found in catches only in August-October (Table 1). All fish, selected for dietary analysis, were

immediately frozen at a temperature of -20°C for further examination.

Laboratory analysis

After thawing, round gobies were measured to a total length (TL, nearest mm), weighted with an accuracy of ± 0.01 g and sorted according 50 mm size groups (Taraborelli & Schaner 2002; Barton et al. 2005): TL <50 mm, 50-99 mm, 100-149 mm, 150-199 mm and ≥ 200 mm. If available, 10-15 randomly selected individuals were analyzed in each size group during a month period.

The alimentary tract from esophagus to anus of each examined specimen was removed, weighed while full and re-weighed when empty (± 0.001 g) to obtain wet weight of the gut content. Feeding objects were identified to the lowest possible taxon, measured and counted under a stereomicroscope. Crushed mollusk shells and hulls of crustaceans were reconstructed from the particles whenever possible to gather taxonomically identifiable and measurable

Table 1

The total number of analyzed female (F), male (M) and juvenile (J) fish, percentage of empty guts and gonad maturity stages of matured round gobies captured during different months of the study

Month	Sex	No. of specimens	Empty guts		Gonad maturity stages (%)				
			No.	%	I	II	III	IV	V
May	F	40	3	7.5	-	-	40	60	-
	M	4	2	50	-	-	75	25	-
	J	-	-	-	-	-	-	-	-
June	F	27	2	5.4	-	-	44.4	55.6	-
	M	8	3	37.5	-	-	62.5	37.5	-
	J	-	-	-	-	-	-	-	-
July	F	35	4	12.1	-	8.6	11.4	20	60
	M	8	3	37.5	-	-	37.5	50	12.5
	J	-	-	-	-	-	-	-	-
August	F	20	4	20	-	55	15	-	30
	M	32	1	3.1	-	21.9	3.1	-	75
	J	26	1	3.8	-	-	-	-	-
September	F	8	2	25	-	100	-	-	-
	M	18	2	11.1	-	66.7	-	-	33.3
	J	22	2	9.5	-	-	-	-	-
October	F	13	2	15.4	-	100	-	-	-
	M	27	4	14.8	-	100	-	-	-
	J	24	1	4.2	-	-	-	-	-

parts of the ingested prey. In the case of a high digestion level, the number of consumed soft-bodied organisms found in posterior guts was determined based on characteristic structural elements such as mandibles and chaetae of polychaetes or telsons, legs and eyes of crustaceans (Skóra & Rzeznik 2001), considering their body length as an average length from all measured individuals of certain prey taxa found in the gut contents of dissected fish. Wet weight of each feeding object was determined using length-weight regression functions (Rumohr et al. 1987). A total of 312 round gobies were analyzed and 36 individuals with empty guts were excluded from the data analysis.

Variation in gonad maturity stages was examined monthly in order to follow the reproduction cycle. Sex of round gobies was determined visually according to the shape of urogenital papillae (Charlebois et al. 1997) and dissected gonads, while small individuals with indeterminable sex were considered as juveniles. Gonads were weighed (± 0.001 g) and their maturity was evaluated under a stereomicroscope using the following descriptive five-stage scale (Tomczak & Sapota 2006).

Gonads of male round gobies were described according the following maturity scale:

- I. Juvenile;
- II. Clearly visible, light beige or slightly grey, not too supple;
- III. Clearly visible, light beige color, but darker than in stage 2, supple;
- IV. Clearly visible, darker than in stage 3, beige, grey-beige to grey with possible red staining on the edges, supple, engorged;
- V. Spent (gonad empty).

Since female round gobies are batch spawners and their gonads contain eggs at higher and earlier developmental stages, maturity was determined according to the fraction of eggs at a higher developmental stage. Evaluation of female eggs' maturity was performed using classification suggested by Tomczak & Sapota 2006:

- I. Poorly developed, yellow or pink, not clearly visible, sometimes difficult to determine the sex;
- II. Small, grains visible, ovaries transparent;

- III. Ovaries yellow or reddish, eggs not transparent, first hydrated oocytes;
- IV. Very well-developed grains, eggs transparent, ovaries yellow or reddish, leakage stage;
- V. Ovary almost empty with single egg grains, ovaries flabby, reddish.

Calculations and statistical analysis

The index of stomach fullness (*ISF*) (Hyslop 1980) was calculated for individuals >100 mm using the following formulas:

$$W_{prey} = W_{stomach\ full} - W_{stomach\ empty} \quad (1)$$

where: W_{prey} = weight of the prey items (g), $W_{stomach\ full}$ = weight of the full stomach (g) and $W_{stomach\ empty}$ = weight of the stomach without prey items (g) and

$$ISF = \frac{W_{prey}}{W} \times 100 \quad (2)$$

where: W = weight of the fish (g).

Contribution of each taxon in the diet of differently sized round gobies was determined using the Index of Relative Importance (*IRI*) (Pinkas 1971):

$$IRI = (\%N + \%W) \times \%F \quad (3)$$

where: %N = relative abundance; %W = weight; %F = frequency of occurrence of feeding objects in all examined alimentary tracts.

For multivariate data analysis and graphical presentation, feeding objects were classified into 12 prey categories: *Macoma balthica*, *Mytilus trossulus*, Cladocera, Copepoda, Ostracoda, Amphipoda (*Gammarus* sp. and *Corophium volutator* combined), *Crangon crangon*, *Saduria entomon*, *Balanus improvisus*, Polychaeta and Pisces. Rarely occurring food items such as *Cerastoderma lamarcki*, *Mya arenaria*, *Hydrobia ulvae* and *Neomysis integer* were pooled and considered as other prey. Non-metric multidimensional scaling (NMDS) analysis was employed to demonstrate dissimilarities in the diet composition among round gobies belonging to different size groups. Permutational one-factorial ANOVA (PERMANOVA) (Anderson 2001) with a random subset of 999 permutations was used to

test the influence of season on the diet of each size group individually. Prior to the statistical analyses, the data on biomass of all 12 major prey categories found in the examined alimentary tracts were standardized by total gut content biomass and square root-transformed to reduce the effects of outliers. Similarity matrices were conducted using the Bray-Curtis similarity coefficient. Multivariate analyses were performed using PRIMER v. 6 software (Clarke & Gorley 2006). Seasonal variation in the diet of round goby was analyzed further by using weight percentage (%W) contribution of different prey taxa (Hyslop 1980). All samples were combined according to spring (May), summer (June-August) and autumn (September-October) seasons.

Results

Feeding activity

Three reproduction periods were distinguished according to the dominant gonad maturity stages: pre-spawning (III and IV maturity stages) took place in May and June, the peak of spawning event (V maturity stage) was recorded in July and August, while September and October (II maturity stage) were considered as a post-spawning period (Table 1). The reproduction period had a major impact on feeding activity of male round gobies, while females were less affected. Feeding activity increased from pre-spawning (May) to post-spawning (September) four times in females, whereas it was more constant in males. The lowest values of the stomach fullness index for male round gobies were recorded in July, before and during the spawning (Fig. 1).

Seasonal and ontogenetic variation of the diet composition

The NMDS analysis demonstrated that diet composition of <50 mm and 50-99 mm round gobies considerably differed from the ration of larger fish (Fig. 2). The most important feeding objects for <100 mm round gobies were ostracods, cladocerans, copepods, amphipods and small specimens of *M. trossulus*. Larger (≥ 100 mm) individuals consumed mainly bivalve mollusks *M. balthica* and *M. trossulus*, polychaetes and fish with a secondary importance of crustaceans: amphipods, brown shrimps

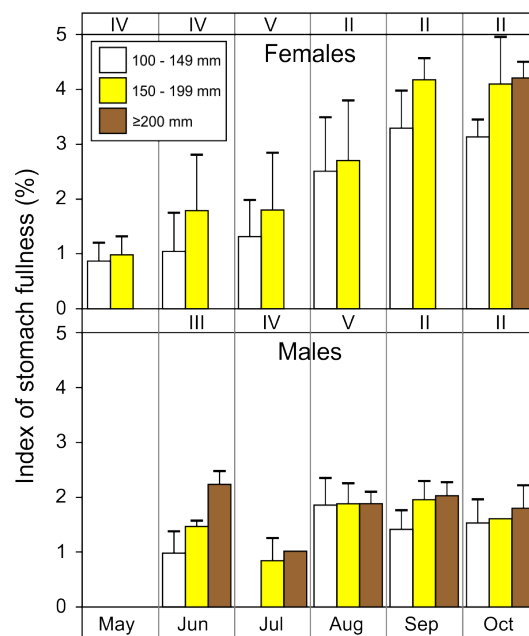


Figure 1

Changes in the index of stomach fullness (mean \pm standard deviation) values and dominant gonad maturity stages of differently sized female and male round gobies during May-October

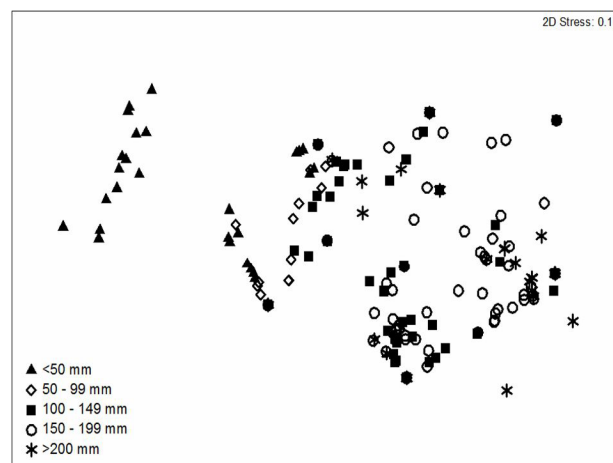


Figure 2

NMDS ordination plot of biomass of prey items, found in the gut contents of differently sized round gobies

(*C. crangon*), barnacles (*B. improvisus*), isopods (*S. entomon*) and mysids (*N. integer*) (Table 2).

As indicated by PERMANOVA, prey composition of <50 mm, 50-99 mm and ≥ 200 mm round gobies was relatively constant in time, while 100-149 mm and 150-199 mm individuals had more variable

Table 2

Diet composition (%IRI) of differently sized round gobies according to relative abundance (%N), weight (%W) and frequency of occurrence (%F) of consumed prey items. Values are derived from pooled data of the whole May-October period; n - the number of individuals with non-empty guts, analyzed within each size group.

Prey items	Size groups of round goby (TL)																			
	<50 mm				50-99 mm				100-149 mm				150-199 mm				≥200 mm			
	n=42				n=27				n=90				n=86				n=31			
	%N	%W	%F	%IRI	%N	%W	%F	%IRI	%N	%W	%F	%IRI	%N	%W	%F	%IRI	%N	%W	%F	%IRI
Mollusks	1.9	47.0	6.1	7.2	17.3	24.7	20.1	12.3	43.9	33	28.4	38.8	45.9	50.9	33.8	55.1	64.9	84.5	47.2	77.3
<i>Macoma balthica</i>	0	0	0	0	0	0	0	0	6.7	17.4	7.8	9.5	17.8	46.2	19.8	38.7	36.7	78.5	32.3	67.2
<i>Mytilus trossulus</i>	1.9	47.0	6.1	7.2	14.0	17.9	14.5	5.6	35.7	9.3	17.4	29.2	27.6	1.8	12.8	16.4	28.2	6.0	14.9	10.1
<i>Cerastoderma lamarcki</i>	0	0	0	0	0	0	0	0	1.2	4.6	2.8	<0.1	0.4	1.3	0.9	<0.1	0	0	0	0
<i>Mya arenaria</i>	0	0	0	0	0	0	0	0	0.3	1.7	0.4	0.1	0.1	1.6	0.3	<0.1	0	0	0	0
<i>Hydrobia ulvae</i>	0	0	0	0	3.3	6.8	5.6	6.7	0	0	0	0	0	0	0	0	0	0	0	0
Crustaceans	98.1	53	93.9	92.8	81.6	48.6	77.1	78.3	31.1	25.3	46.6	24.3	28.3	26.1	36.3	18.2	23.7	9.8	29.9	9.9
Cladocera	33.2	13.4	23.3	26.0	9.6	0.1	8.1	17.3	0	0	0	0	0	0	0	0	0	0	0	0
Copepoda	25.4	9.6	29.7	25.9	19.2	0.3	14.7	1.1	0	0	0	0	0	0	0	0	0	0	0	0
Ostracoda	35.5	14.1	29.5	30.6	15.2	0.2	9.9	0.4	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gammarus</i> sp.	4.0	15.9	11.4	10.3	34.9	45.7	38.1	58.9	7.2	0.7	8.4	7.4	4.9	0.5	6.6	1.4	2.6	0.2	5.3	1.0
<i>Corophium volutator</i>	0	0	0	0	0	0	0	0	8.2	1.0	10.3	4.2	1.5	0.2	2.1	0.5	0.3	<0.1	2.9	0.6
<i>Crangon crangon</i>	0	0	0	0	0	0	0	0	2.9	16.1	5.5	6.5	2.6	14.1	6.4	3.1	0	0	0	0
<i>Neomysis integer</i>	0	0	0	0	0	0	0	0	3.9	0.3	7.9	1.5	0.2	<0.1	0.3	<0.1	0.5	<0.1	1.3	<0.1
<i>Saduria entomon</i>	0	0	0	0	0	0	0	0	0.2	4.0	0.4	0.1	0.4	8.9	2.0	1.2	1.0	7.9	3.0	1.0
<i>Balanus improvisus</i>	0	0	0	0	2.7	2.3	6.3	0.6	8.7	3.2	14.1	4.6	18.7	2.4	18.9	12.0	19.3	1.7	17.4	7.4
Polychaetes	0	0	0	0	1.1	26.7	2.8	9.4	23.9	31.8	20.4	34.4	21.5	12.0	16.5	21.3	8.7	3.3	15.0	11.8
Pisces	0	0	0	0	0	0	0	0	1.1	9.9	4.6	2.5	4.3	11	13.4	5.4	2.7	2.4	7.9	1.0
<i>Osmerus eperlanus</i>	0	0	0	0	0	0	0	0	0.2	2.3	0.9	2.2	2.9	8.5	8.7	1.2	1.6	1.7	4.8	0.7
<i>Gasterosteus aculeatus</i>	0	0	0	0	0	0	0	0	0.7	7.1	2.8	0.2	1.1	2.3	2.8	4.0	0.6	0.6	1.8	0.3
<i>N. melanostomus</i>	0	0	0	0	0	0	0	0	0.2	0.5	0.9	0.1	0.3	0.2	1.9	0.2	0.5	0.1	1.3	0

diet, which used to change depending on the season (Table 3).

In spring, diet composition of round gobies was the least diverse and consisted mainly of mollusks and crustaceans. Importance of mollusks increased with a fish size and represented 56.2% and 72.5% relative contribution by weight in the diet of individuals from 100-149 mm and 150-199 mm size groups, respectively; the rest of the diet consisted of amphipods (2.0% and 1.6%), brown shrimps (34.8% and 13.4%), polychaetes (6.3% and 2.4%), barnacles (0.7% and 1.6%) and fish (0.0% and 8.5%). The largest

round gobies (≥200 mm) fed exclusively on bivalves *M. balthica* (90.9%) and *M. trossulus* (9.1%) (Fig. 3A).

In summer, <50 mm round gobies consumed *M. trossulus* (43.0%), amphipods (22.1%), cladocerans (13.4%), ostracods (10.8%) and copepods (10.7%), while larger individuals (50-99 mm) preyed mainly on amphipods (60.9%), polychaetes (23.3%) and bivalve mollusks (10.2%) with negligible contribution of barnacles (1.4%), copepods (0.5%), cladocerans (<0.1%), ostracods (<0.1%) and other prey items (3.5%) in their diet (Fig. 3B). Bivalve mollusks (51.0%), ostracods (17.4%), cladocerans

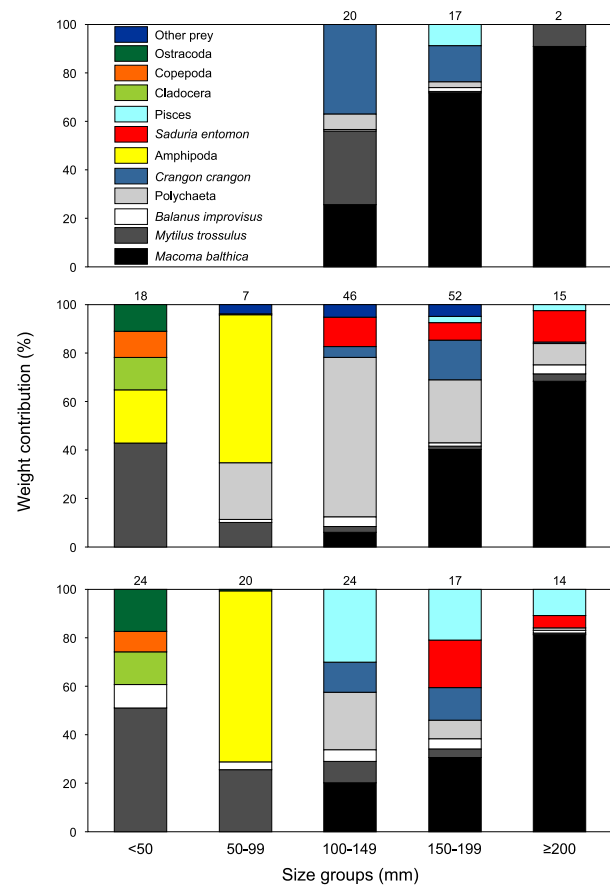
(13.5%), barnacles (9.7%) and copepods (8.4%) were the most important feeding objects in the diet of <50 mm round gobies in autumn, while the bulk of 50-99 mm fish ration was composed of amphipods (70.4%), bivalve mollusks (25.6%) and barnacles (3.2%) with a minor contribution of ostracods (0.5%), cladocerans (0.2%) and copepods (<0.1%) (Fig. 3C).

Diet composition of intermediately sized (100-200 mm) individuals was different in summer compared to the spring. Importance of mollusks and crustaceans declined, whereas importance of polychaetes increased sharply. In June-August, polychaetes represented 65.7% and 26.0% in the diet of fish from 100-149 mm and 150-199 mm size groups, while in autumn consumption rates of polychaetes declined to 23.6% and 7.5%, respectively. Contribution of bivalve mollusks (8.7% and 41.7%), crustaceans (20.6% and 25%) and other prey items (5.0% and 4.8%) was also significant, while fish prey was less important (0.0% and 2.5%) (Fig. 3B).

Table 3

PERMANOVA of season effect on round goby diet composition (by weight contribution of 12 prey categories) within each size group

Size group (mm)	Source	df	SS	MS	Pseudo-F	P
<50	Season	1	2894.5	2884.1	1.137	0.304
	Residual	42	59312	2478.5		
	Total	43	65217			
50-99	Season	1	2531	2272	1.132	0.270
	Residual	27	38420	2089.7		
	Total	28	41021			
100-149	Season	2	15324	8270.5	2.457	0.012
	Residual	90	<0.001	3221.8		
	Total	92	<0.001			
150-199	Season	2	11314	6246.5	2.146	0.047
	Residual	86	<0.001	2987.2		
	Total	88	<0.001			
≥200	Season	2	10951	5641.3	1.691	0.183
	Residual	31	<0.001	3147.5		
	Total	33	<0.001			

**Figure 3**

Mass percentage diet composition of the round goby during spring (A), summer (B) and autumn (C) seasons. Presented weight contributions (%) are averages of pooled values of female and male individuals during each season. The numbers above columns indicate sample sizes.

Changes in the diet composition of intermediately sized round gobies in autumn were mostly induced by lowered consumption of polychaetes and increased importance of *M. trossulus* (8.9% and 3.3%) and fish (30.0% and 21.0%). The overall consumption rates of mollusks (29.0% and 34.1%) and crustaceans (17.3% and 37.4%) remained almost the same as those determined during summer season (Fig. 3C).

The most common prey item for round gobies ≥200 mm was infaunal bivalve mollusk *M. balthica*, which constituted 90.9%, 68.4% and 81.2% in the diet during spring, summer and autumn, respectively. Other prey items, including epibenthic bivalves, crustaceans and fish were consumed less actively (Figs. 3A-C).

Discussion

Feeding activity of round gobies varied in relation to the reproductive cycle. Values of the stomach fullness index for females were relatively low from May to July, because before and during the spawning they usually spend most of the time near the nests, protected by males or spawn (MacInnis & Corkum 2000), resulting in suppressed foraging at that time. After spawning, females immediately leave reproduction sites (Meunier et al. 2009) and begin to feed actively, reaching the highest values of feeding activity during the post-spawning period. Such behavioral adaptation allows females to accumulate sufficient amounts of energy to produce large quantities of eggs for multiple batch spawnings. Males had lower gut fullness compared to females. Contrary to females, males are sedentary and guard their nests from the beginning of reproduction (Corkum et al. 1998). During the breeding, male round gobies do not feed or their diet is very poor, composed of eggs from their own or nearby located nests and organisms found in a very close vicinity of the nesting site. In July, during the peak of spawning, feeding activity of male round gobies was the lowest due to their immobility induced by nest guarding and parental care behavior. Feeding activity between male and female individuals varies at different sites, e.g. Thompson and Simon (2014) reported females feeding less actively than males in Lake Erie, while in the Black Sea, feeding activity was higher for females (Rosca & Surugiu 2010). Our findings revealed that feeding activity for both female and male round gobies reaches the peak during the post-spawning period, suggesting that benthic fauna is under particular pressure at that time, specifically September-October.

Many studies emphasize the impact of round goby in the food web through predation on mollusks. The contribution of mollusks in the diet typically increases with increasing fish size (Skora & Rzeznik 2001; Raby et al. 2010; Brush et al. 2012), because gape height and width are the main limiting factors, determining the type and size of consumed prey (Ray & Corkum 1997). Gape limited juvenile individuals prefer soft-bodied prey, such as zooplanktonic or meiobenthic organisms and small amphipods, and avoid hardly digestible mollusks (Phillips et al. 2003; Hayden & Miner 2009). Our study proves the

consistency of these patterns. The most important feeding object, ingested by >100 mm individuals was *M. balthica*, however, we also determined seasonal variability of prey taxa consumed by differently sized round gobies. The lack of new data on prey availability makes the comprehensive evaluation of round goby feeding preferences difficult. According to previous studies from the round goby pre-invasion period, the infaunal soft bottom benthic community was dominated by mollusks (79% of the total zoobenthos biomass), crustaceans (11%) and polychaetes (9.8%) in the near-shore (4-9 m) zone, representing our study site (Bubinas & Repečka 2003). Availability of epifaunal mollusks in the study area changes seasonally, as there is no permanent population of *M. trossulus* on the hydrotechnical constructions of harbor moles. Abundance of *M. trossulus* at the study site reaches the peak during autumn season, after recruitment of mussel spat (Kautsky 1982). Increment of juvenile (2-3 mm) blue mussel abundance was particularly reflected in the diet of 100-200 mm fish; likely round gobies of this size prefer epifaunal mollusks, while soft bottom species, burrowed deeply into the sediments are of limited access. We also determined relatively high weight contribution of *M. trossulus* in the diet of juvenile individuals, however, it occurred only in negligible number of analyzed stomachs. In contrast, the largest individuals (≥ 200 mm) were less dependent on seasonal variation in the availability of small epibenthic mollusks and preferred *M. balthica* even in autumn. Results from previously conducted studies (French & Jude 2001; Raby et al. 2010; Brush et al. 2012) also showed that the ration of large gobies is the least variable and mainly consists of bivalve mollusks. Moreover, large fish are capable of ingesting bivalves of a wider size range by swallowing entire mollusks or crushing them with pharyngeal teeth (Ghedotti et al. 1995).

Round gobies are known to forage on the most abundant components of benthic community, which may change seasonally (Skora & Rzeznik 2001; Banaru & Harmelin-Vivien 2009). Diet composition of intermediately sized round gobies was the most variable during the course of present research. In spring, round gobies 100-200 mm fed mainly on mollusks and barnacles, with a large portion of brown shrimps in the gut contents. The highest relative importance of *C. crangon* in the

diet was observed during spring season, because brown shrimps migrate from deeper areas and accumulate in the coastal waters at that time (Lapinska & Szaniawska 2006). During summer season, intermediately sized round gobies shifted their diet from mollusks to polychaetes. The highest abundance of polychaetes in the guts of analyzed fish was recorded in midsummer, when the population of *Hediste diversicolor* was dominated by small, newly hatched individuals that stay close to the sediment surface (Marty & Retiere 1999) and represent easily accessible prey for benthivorous fish. In autumn, relative importance of polychaetes declined considerably in the diet of 100-200 mm individuals, whereas importance of juvenile *M. trossulus* and fish increased. In September and October, round gobies preyed on fish more frequently compared to spring or summer and their main fish prey was represented by juvenile smelts, that are concentrating in the coastal waters of the Baltic Sea in autumn (Ustups et al. 2003). Small juvenile round gobies also occurred occasionally in the guts of large specimens and these findings confirmed cannibalistic feeding habits of the species, previously determined by French and Jude (2001) and Carman et al. (2006).

The presented study demonstrated ability of round goby to change feeding patterns depending on the body size, sex, stage of the reproduction cycle and changing seasonal availability of preferred prey items. Constantly increasing abundance of this invasive fish is a serious threat to the Baltic Sea, which may cause significant abundance reduction or even extinction of various native invertebrate species (Kuhns & Berg 1999; Barton et al. 2005; Krakowiak & Pennuto 2008). Abundance of epifaunal bivalve *M. trossulus* and other benthic invertebrates, living in association with its colonies in the Lithuanian coastal waters of the Baltic Sea declined dramatically after round goby invasion (Stupelytė 2014). Round goby predation on newly settled blue mussels has significant potential to reduce its recruitment success, especially in areas where space on suitable hard substrates is limited. Further, more comprehensive field researches and laboratory experiments are necessary to prove negative effects of this invader on both infaunal and epifaunal zoobenthic communities.

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References

- Almqvist G., Strandmark A.K. & Apellberg M. (2010). Has the invasive round goby caused new links in Baltic food webs? *Environ. Biol. Fishes* 89(1):79-93. DOI: 10.1007/s10641-010-9692-z.
- Anderson M.J. (2001). A new method for non-parametric multivariate analysis of variance. *Aust. J. Ecol.* 26: 32-46.
- Balshine S., Verma A., Chant V. & Theysmeyer T. (2005). Competitive Interactions between Round Gobies and Logperch. *J. Gt. Lakes Res.* 31: 68-77. DOI: 10.1016/S0380-1330(05)70238-0.
- Barton D.R., Johnson R.A., Campbell L., Petruniak J. & Patterson M. (2005). Effects of Round gobies (*Neogobius melanostomus*) on dreissenid mussels and other invertebrates in Eastern Lake Erie, 2002-2004. *J. Gt. Lakes Res.* 31(2): 252-261. DOI: 10.1016/S0380-1330(05)70318-X.
- Björklund M. & Almqvist G. (2010). Rapid spatial genetic differentiation in an invasive species, the round goby *Neogobius melanostomus* in the Baltic Sea. *Biol. Invasions* 12(8): 2609-2618. DOI: 10.1007/s10530-009-9669-z.
- Borcherding J., Dolina M., Heermann L., Knutzen P., Kruger S., Marten S., Treck R. & Gertzen J. (2013). Feeding and niche differentiation in three invasive gobies in the Lower Rhine, Germany. *Limnologica* 43: 49-58. DOI: 10.1016/j.limno.2012.08.003.
- Borza P., Erös T. & Oertel N. (2009). Food Resource Partitioning between Two Invasive Gobiid Species (Pisces, Gobiidae) in the Littoral Zone of the River Danube, Hungary. *Int. Rev. Hydrobiol.* 94(5): 609-621. DOI: 10.1002/iroh.200911134.
- Brandner J., Auerswald K., Cerwenka A. F., Schlieven U.K. & Geist J. (2013). Comparative feeding ecology of invasive Ponto-Caspian gobies. *Hydrobiologia* 703: 113-131. DOI: 10.1007/s10750-012-1349-9.
- Brush J.M., Fisk A.T., Hussey N.E., Johnson T.B. (2012). Spatial and seasonal variability in the diet of round goby (*Neogobius melanostomus*): stable isotopes indicate that stomach content overestimate the importance of dreissenids. *Can. J. Fish. Aquat. Sci.* 69(3): 573-586. DOI: 10.1139/f2012-001.
- Bubinas A. & Repečka M. (2003). Distribution of zoobenthos in the bottom sediments of the near – shore zone of the Baltic Sea in Nida - Klaipėda stretch. *Acta Zool. Lit.* 13: 125-134.

- DOI: 10.1080/13921657.2003.10512556.
- Bzoma S. (1998). The contribution of round goby (*Neogobius melanostomus* Pallas, 1811) to the food supply of cormorants (*Phalacrocorax carbo* Linnaeus, 1758) feeding in the Puck Bay. *Bull. Sea Fisheries Inst.* 2: 39-47.
- Carman C.M., Janssen J. & Jude D.J. (2006). Diel interactions between prey behaviour and feeding in an invasive fish, the round goby, in a North American river. *Freshwater Biol.* 51: 74-755. DOI: 10.1111/j.1365-2427.2006.01527.x.
- Charlebois P.M., Marsden J.E., Goettel R.G., Wolfe R.K., Jude D.J. & Rudnika S. (1997). The round goby, *Neogobius melanostomus* Pallas, a review of European and North American literature. Illinois-Indiana Sea Grant Program and Illinois Natural History Survey. INHS Special Publication No. 20. 76 pp.
- Clarke K.R. & Gorley R.N. (2006). Primer v. 6: User manual/tutorial, PRIM ER-E Ltd., Plymouth U.K., 192 pp.
- Copp G.H., Bianco P.G., Bogutskaya N.G., Eros T., Falka I., Ferreira M.T., Fox M.G., Freyhof J., Gozlan R.E., Grabowska J., Kovač V., Moreno-Amich R., Naseka A.M., Penaz M., Povž M., Przybylski M., Robillard M., Russell I. C., Stakėnas S., Šumer S., Vila-Gispert A. & Wiesner C. (2005). To be, or not to be, a non-native freshwater fish? *J. Appl. Ichthyol.* 21(4): 242-262. DOI: 10.1111/j.1439-0426.2005.00690.x.
- Corkum L.D., MacInnis A.J. & Wickett R. G. (1998). Reproductive Habits of Round Gobies. *Great Lakes Research Review* 3(2):13-20.
- Corkum L.D., Sapota M.R. & Skóra K.E. (2004). The round goby, *Neogobius melanostomus*, a fish invader on both sides of the Atlantic Ocean. *Biol. Invasions* 6(2): 173-181. DOI: 10.1023/B:BINV.0000022136.43502.db.
- French J.R.P. & Jude D.J. (2001). Diets and Diet Overlap of Nonindigenous Gobies and Small Benthic Native Fishes Co-inhabiting the St. Clair River, Michigan. *J. Gt. Lakes Res.* 27(3): 300-311.
- Ghedotti M.J., Smihula J.C. & Smith G.R. (1995). Zebra Mussel Predation by Round Gobies in the Laboratory. *J. Gt. Lakes Res.* 21(4): 665-669. DOI: doi:10.1016/S0380-1330(95)71076-0.
- Hayden T.A. & Miner J.G. (2009). Rapid dispersal and establishment of a benthic Ponto-Caspian goby in Lake Erie: diel vertical migration of early juvenile round goby. *Biol. Invasions* 11(8): 1767-1776. DOI: 10.1007/s10530-008-9356-5.
- Hyslop E.J. (1980). Stomach contents analysis-a review of methods and their application. *J. Fish Biol.* 17: 411-429. DOI: 10.1111/j.1095-8649.1980.tb02775.x.
- Jude D.J., Janssen J. & Crawford G. (1992). Establishment of Gobiidae in the Great Lakes basin. *Can. J. Fish. Aquat. Sci.* 49(2): 416-421.
- Kakareko T., Zbikowski J. & Zytkowicz J. (2005). Diet partitioning in summer of two syntopic neogobiids from two different habitat of the lower Vistula River, Poland. *J. Appl. Ichthyol.* 21: 292-295. DOI: 10.1111/j.1439-0426.2005.00683.x.
- Karlson A.M.L., Almqvist G., Skóra K.E. & Appelberg M. (2007). Indications of competition between non-indigenous round goby and native flounder in the Baltic Sea. *ICES J. Mar. Sci.* 64: 479-486. DOI: 10.1093/icesjms/fsl049.
- Kautsky N. (1982). Quantitative studies of gonad cycle, fecundity, reproductive output and recruitment in a Baltic *Mytilus edulis* population. *Mar. Biol.* 68: 143-160. DOI: 10.1007/BF00397601.
- Kornis M.S., Mercado-Silva M. & Vander Zanden M.J. (2012). Twenty years of invasion: a review of round goby *Neogobius melanostomus* biology, spread and ecological implications. *J. Fish Biol.* 80: 235-285. DOI: 10.1111/j.1095-8649.2011.03157.x.
- Krakowiak P.J. & Pennuto C.M. (2008). Fish and Macroinvertebrate Communities in Tributary Streams of Eastern Lake Erie with and without Round Gobies (*Neogobius melanostomus*, Pallas 1814). *J. Gt. Lakes Res.* 34: 675-689. DOI: <http://dx.doi.org/10.3394/0380-1330-34.4.675>.
- Kuhns L.A. & Berg M.B. 1999. Benthic Invertebrate Community Responses to Round Goby (*Neogobius melanostomus*) and Zebra Mussel (*Dreissena polymorpha*) Invasion in Southern Lake Michigan. *J. Gt. Lakes Res.* 25(4): 910-917. DOI:10.1016/S0380-1330(99)70788-4.
- Lapinska E. & Szaniawska A. (2006). Seasonal variations in the occurrence of the prawns *Crangon crangon* (L, 1758), *Palaemon adspersus* (Rathke, 1837), and *Palaemon elegans* (Rathke, 1837) in the littoral zone of the Gulf of Gdansk. *Crustaceana* 79(6): 95-110.
- Lundström K., Hjerne O., Lunneryd S.G. & Kallson O. (2010). Understanding the diet composition of marine mammals: grey seals (*Halichoerus grypus*) in the Baltic Sea. *ICES J. Mar. Sci.* 67(6): 1230-1239. DOI: 10.1093/icesjms/fsq022.
- MacInnis A.J. & Corkum L.D. (2000). Fecundity and Reproductive season of the Round Goby *Neogobius melanostomus* in the Upper Detroit River. *Trans. Am. Fish. Soc.* 129: 136-144. DOI: 10.1577/1548-8659(2000)129<0136:FARSOT>2.0.CO;2.
- Marty R. & Retiere C. (1999). Larval-to-juvenile mobility activities of a holobenthic species, *Nereis diversicolor* (O.F. Müller) (Polychaeta: Nereidae) their involvement in recruitment. *Bull. Mar. Sci.* 65(3): 761-774.
- Meunier B., Yvano S., Ahmed S. & Corkum L.D. (2009). First

- documentation of spawning and nest guarding in the laboratory by the invasive fish, the round goby (*Neogobius melanostomus*). *J. Gt. Lakes Res.* 35: 608–612. DOI:10.1016/j.jglr.2009.08.012.
- Ojaveer H. (2006). The round goby *Neogobius melanostomus* is colonising the NE Baltic Sea. *Aquat. Invasions* 1(1): 44–45. DOI 10.3391/ai.2006.1.1.11.
- Olenin S. & Daunys D. (2004). Coastal typology based on benthic biotope and community data: The Lithuanian case study. *Coastline Reports* 4: 65–83.
- Olenin S., Daunys D. & Labanauskas V. (1996). Classification principles of the Baltic Sea Lithuanian coastal benthic biotopes. *Geografijos metraštis* 29: 218–231 (in Lithuanian).
- Pennuto C.M., Krakowiak P.J. & Janik C.E. (2010). Seasonal abundance, diet, and energy consumption of round gobies (*Neogobius melanostomus*) in Lake Erie tributary streams. *Ecol. Freshw. Fish* 19(2): 206–215. DOI: 10.1111/j.1600-0633.2010.00405.x.
- Phillips E.C., Washek M.E., Hertel A.W. & Niebel B.M. (2003). The Round Goby (*Neogobius melanostomus*) in Pennsylvania Tributary Streams of Lake Erie. *J. Gt. Lakes Res.* 29(9): 34–40. DOI:10.1016/S0380-1330(03)70413-4.
- Pinkas L., Oliphant M.S. & Iverson L.K.I. (1971). Food habits of albacore, bluefin tuna, and bonito in California waters. State of California the Resources Agency Department of fish and Game. *Fish Bulletin* 152, 105 pp.
- Płażochki D., Kobak J. & Kakareko T. (2012). First report on the importance of alien gobiids in the diet of native piscivorous fishes in the lower Vistula River (Poland). *Oceanol. Hydrobiol. Stud.* 41(2): 83–89. DOI: 10.2478/s13545-012-0020-4.
- Poláčik M., Janač M., Jurajda P., Adamek Z., Ondračková M., Trichkova T. & Vassilev M. (2009). Invasive gobies in the Danube: invasion success facilitated by availability and selection of superior food resources. *Ecol. Freshw. Fish* 18: 640–649. DOI: 10.1111/j.1600-0633.2009.00383.x.
- Pūtys Ž. & Zarankaitė J. (2010). Diet of the Great Cormorant (*Phalacrocorax carbo sinensis*) at the Juodkrantė colony, Lithuania. *Acta Zool. Lit.* 20: 179–189. DOI: 10.2478/v10043-010-0031-6.
- Raby G.D., Gutowsky L.F.G. & Fox M.G. (2010). Diet composition and consumption rate in round goby (*Neogobius melanostomus*) in its expansion phase in the Trent River, Ontario. *Environ. Biol. Fishes* 89(2): 143–150. DOI: 10.1007/s10641-010-9705-y.
- Ray W.J. & Corkum L.D. (1997). Predation of zebra mussels by round gobies, *Neogobius melanostomus*. *Environ. Biol. Fishes* 50: 267–273. DOI: 10.1023/A:1007379220052.
- Rakauskas V., Bacevičius E., Pūtys Ž., Ložys L. & Arbačiauskas K. (2008). Expansion, feeding and parasites of the round goby, *Neogobius melanostomus* (Pallas, 1811), a recent invader in the Curonian Lagoon, Lithuania. *Acta Zool. Lit.* 18(3): 180–190. DOI: 10.2478/v10043-008-0030-z.
- Rakauskas V., Pūtys Ž., Dainys J., Lesutienė J., Ložys L. & Arbačiauskas K. (2013). Increasing population of the invader round goby *Neogobius melanostomus* (Actinopterygii: Perciformes: Gobiidae), and its trophic role in the Curonian lagoon, SE Baltic Sea. *Acta Ichthyol. Pisc.* 43(2): 95–108. DOI: 10.3750/AIP2013.43.2.02.
- Rosca I. & Surugiu V. (2010). Feeding ecology of some benthic fish species the Romanian Black Sea coast (Egeia-Eforie nord area). *Analele Științifice ale Universității „Al. I. Cuza” Iași, s. Biologie animală*, Tom LVI: 249–256.
- Rumohr H., Brey T. & Ankar S. (1987). A compilation of biometrics conversion factors for benthic invertebrates of the Baltic Sea. Baltic Marine Biologists Publication No 9. 56 pp.
- Sapota M.R. & Skóra K.E. (2005). Spread of alien (non-indigenous) fish species *Neogobius melanostomus* in the Gulf of Gdansk (south Baltic). *Biol. Invasions* 7(2): 157–164. DOI: 10.1007/s10530-004-9035-0.
- Sapota M.R. (2004). The round goby (*Neogobius melanostomus*) in the Gulf of Gdansk - a species introduction into the Baltic Sea. *Hydrobiologia* 514: 219–224. DOI: 10.1023/B:hydr.0000018221.28439.ae.
- Skóra K.E. & Rzeźnik J. (2001). Observations on diet composition of *Neogobius melanostomus* Pallas 1811 (Gobiidae, Pisces) in the Gulf of Gdansk (Baltic Sea). *J. Gt. Lakes Res.* 27(3): 290–299. DOI: 10.1016/S0380-1330(01)70644-2.
- Stupelytė A. (2014). *Distribution and biomass dynamics of blue mussel (*Mytilus edulis trossulus*, (Gould, 1850)) in the Lithuanian coastal waters of the Baltic Sea.* Unpublished master thesis, Klaipėda University, Klaipėda, Lithuania.
- Taraborelli A.C. & Schaner T. (2002). Diet of Round Goby in the Bay of Quinte, Lake Ontario. Lake Ontario communities and fisheries: 2001 annual report of the Lake Ontario management unit: 106–109.
- Thompson H.A. & Simon T.P. (2014). Diet shift response in round goby, *Neogobius melanostomus*, based on size, sex, depth and habitat in the western basin of Lake Erie. *J. Appl. Ichthyol.* 30: 955–961. DOI: 10.1111/jai.12441.
- Tomczak M.T., Sapota M.R. (2006). The fecundity and gonad development cycle of the round goby (*Neogobius melanostomus*, Pallas 1811) from the Gulf of Gdansk. *Oceanol. Hydrobiol. Stud.* 35(4): 353–367.
- Ustups D., Urtans E., Minde A. & Uzars D. (2003). The structure and dynamics of fish communities in the Latvian coastal zone (Pape-Pērkone), Baltic Sea. *Acta Universitatis*

Latviensis 662: 33-44.

- Vašek M., Všetickova L., Roche K. & Jurajda P. (2014). Diet of two invading gobiid species (*Proterorhinus semilunaris* and *Neogobius melanostomus*) during the breeding and hatching season: no field evidence of extensive predation on fish eggs and fry. *Limnologica* 46: 31-36. DOI: 10.1016/j.limno.2013.11.003.
- Walsh M.G., Dittman D.E. & O’Gorman R. (2007). Occurrence of food habits of the round goby in the profundal zone of Southwestern Lake Ontario. *J. Great Lakes Res.* 33: 83-92. DOI: 10.3394/0380-1330(2007)33[83:OAFHOT]2.0.CO;2.
- Wandzel T. (2003). The food and feeding of the round goby (*Neogobius melanostomus* Pallas, 1811) from the Puck Bay and the Gulf of Gdansk. *Bull. Sea Fish. Inst.* 2: 43-51.
- Zolubas T. (2003). New invasive fish species in Lithuanian waters. *Jūra ir aplinka* 2(9): 52-56 (in Lithuanian).

Effect of non-indigenous round goby (*Neogobius melanostomus*) on the native European flounder (*Platichthys flesus*) biomass density in the southern Baltic Sea

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The Harris Mud Crab (*Rhithropanopeus harrisi*)

Food web positioning of a recent coloniser: the North American Harris mud crab *Rhithropanopeus harrisii* (Gould, 1841) in the northern Baltic Sea

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Abstract

Identifying resource use and functional traits of non-indigenous species are promising means to increase the ability to predict ecological consequences of invasions. One of the most widely spread brachyuran crab species, and a recent invader in the northern Baltic Sea, is the North American Harris mud crab, *Rhithropanopeus harrisii* (Gould, 1841). Although spread, establishment, and population structure have been studied for this species, little is known about its role and impact in a new environment. We investigated the position and role of *R. harrisii* in the coastal food web by combining spatial and temporal estimates of trophic position using stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), with short-term food- and habitat-choice experiments. We observed a stable pattern in the trophic position between two environmentally different locations in the Archipelago Sea over the production season. The identification of a natural breakpoint in carbon stable isotopes at the size of 12 mm carapace width suggested a presence of an ontogenetic diet shift, which was confirmed by isotope signatures positioning large *R. harrisii* among secondary consumers and small individuals with primary consumers. The generalist consumer nature of *R. harrisii* and preference for structured habitat was supported by an active habitat and food choice, revealing no preference for prey species (polychaetes versus amphipod), but selection of brown algae over bare mud. To broaden the understanding of the functional role of *R. harrisii*, we combined our findings with other known biological and ecological traits of the species and qualitatively compared these to characteristics of other benthic organisms in the northern Baltic Sea, suggesting a general similarity in traits.

Key words: introduced species, feeding, habitat choice, stable isotopes, biological traits, experiment

Introduction

Human-mediated species introductions are a continuing threat to both terrestrial and marine biodiversity and thus an important part of global change (Strayer 2012; Simberloff et al. 2013; Jeschke et al. 2014). To increase our ability to predict ecological changes due to non-native species introductions, invasion ecology needs to move beyond descriptive cataloguing of establishment and impact studies and to a more mechanistic understanding and quantification of invader effects (Dick et al. 2014; Paterson et al. 2015). The most promising efforts to generalise mechanisms have focused on how non-indigenous species alter communities and ecosystems through resource use (Vitousek 1990; Parker et al. 1999; Dick et al. 2014). Differences in resource use, such as food or habitat, are reflected in practically all of the

29 hypotheses designed to explain invasion success summarised by Catford et al. (2009). For many of these hypothesis, a major factor that influence invasion success is related to the biological characteristics of the invading species and/or species in the recipient community (Catford et al. 2009; Dick et al. 2014). Recent progress in comparisons of species traits between invaders and natives is encouraging in assessing establishment and spread of invaders (Dick et al. 2014), particularly for terrestrial plant species (Van Kleunen et al. 2010) and to some degree for fish (Marchetti et al. 2004), but still remains patchy (Hayes and Barry 2008; Dick et al. 2014). Moreover, characterising and quantifying traits of non-native species to specifically assess impact is also emerging (Dobb et al. 2014; Paterson et al. 2015). In one study, a comparison between marine invasive invertebrates in only one trait, size,

revealed effects on the invading species itself (Grosholz and Ruiz 2003). Gribben et al. (2013) examined several traits in a biogeographic evaluation of the porcelain crab *Petrolisthes elongatus* (H. Milne-Edwards, 1837) while Gothland et al. (2014) described biological traits related to colonisation and distribution Asian shore crab *Hemigrapsus takanoi* Asakura and Watanabe, 2005. The current gap in the use of species' ecological knowledge between invasion ecology and other ecological fields is interesting because trait-based approaches have proven successful for disentangling marine biodiversity-ecosystem functional linkages (Solan et al. 2006), macroecological patterns (Webb et al. 2009), and anthropogenic impacts (Bremner et al. 2006; de Juan et al. 2007; Villéger et al. 2010).

Some mesohaline habitats, particularly in higher latitude areas, are characterized by relatively low species numbers and relatively weak interspecific competition interactions, which provides the opportunity for colonization by non-indigenous species (Cohen and Carlton 1998; Stachowicz et al. 2002). The Baltic Sea has abiotic and biotic conditions seemingly ideal for establishment of introduced species (Paavola et al. 2005; Gollash and Leppäkoski 2007; Leppäkoski et al. 2009). This mainly brackish (salinity 0.5–20) water body is geologically young (< 10,000 years), with a biota consisting of post-glacial immigrants (Bonsdorff 2006; Leppäranta and Myrberg 2009). Native species diversity is low, which results in simple food webs with relatively few links (Bonsdorff and Blomqvist 1993; Elmgren and Hill 1997). To date, 119 non-indigenous species have been found in the Baltic Sea, of which 20 species have been reported in the northern Baltic Sea and the Archipelago Sea area (David et al. 2013), which is the region of focus in this study. One of the more recent invaders in the northern Baltic Sea is the North American Harris mud crab, *Rhithropanopeus harrisi* (Gould, 1841). The species is a small (adult maximum carapace width 25 mm) brachyuran crab that tolerates a wide range of temperatures (4–35 °C) and salinities (0.5 to > 40) (Costlow et al. 1966; Forward 2009). The native range of *R. harrisi* extends along the Atlantic coast of North America from the Gulf of St Lawrence in Canada to Veracruz in the Gulf of Mexico, where it occupies a wide variety of substrates and habitats in river mouths and estuaries (Williams 1984; Fowler et al. 2013). The species was already known in Europe in 1874, probably arriving in ballast water. *R. harrisi* was first found in the southern Baltic Sea in the 1950s,

where it has since become established (Turoboyski 1973; Hegele-Drywa and Normant 2014). In the Archipelago Sea, *R. harrisi* was first recorded in 2009, and it has since established reproducing populations in more than 80 locations within a 30 km radius of the site of initial colonization (Fowler et al. 2013). Apart from the occasional adult Chinese mitten crab (*Eriocheir sinensis* H. Milne-Edwards, 1853) (Anger 1991), there were no other crab species in the northern Baltic Sea prior to the arrival of *R. harrisi* (Ojaveer et al. 2007).

The establishment and spread of *Rhithropanopeus harrisi* globally and in the Baltic Sea is well catalogued, but few studies have assessed the impact of this species on the recipient community and ecosystem. Similar to other non-indigenous crab species (e.g., Goodenough 2010; Brockernhoff and McLay 2011), there is clear evidence of competition between *R. harrisi* and native crabs and benthivorous fishes at sites in Europe and on the west coast of North America (Cohen and Carlton 1995; Zaitsev and Öztürk 2001). The species also alters the local food webs because *R. harrisi* is both a predator on, and prey for, native species (Turoboyski 1973; Cohen and Carlton 1995; Zaitsev and Öztürk 2001; Fowler et al. 2013). *R. harrisi* is an opportunistic omnivore that feeds on a mixture of animals, vegetation, and detritus (Turoboyski 1973; Czerniejewski and Rybczyk 2008; Hegele-Drywa and Normant 2009). As in its native range, the species occupies a diverse array of substrates in the northern Baltic Sea, including: bare soft sediment, stones, decaying reeds (*Phragmites australis* (Cav.) Trin. ex Steud.), brown algae (*Fucus vesiculosus* Linnaeus, 1753) and other vegetation that provide both protection and a high density and rich variety of prey (Fowler et al. 2013; Nurkse et al. 2015).

The overall aim of this study was to investigate the trophic position and role of *Rhithropanopeus harrisi* in the coastal food web of the northern Baltic Sea. We addressed this by combining spatial and temporal estimates of trophic position of *R. harrisi* with short-term habitat- and food choice experiments. More specifically, we wanted to: i) determine ontogenetic differences in trophic position of *R. harrisi*; ii) assess temporal variability of the species' consumer characteristics; and iii) identify whether *R. harrisi* displays an active choice for selected substrates and prey species. We then qualitatively compared the known biological and ecological traits of *R. harrisi* to other benthic organisms in the northern Baltic Sea as a means of evaluating the

effects of introduction of *R. harrisii* on ecosystem structure and functioning.

Acknowledging that the diet of generalist predators rely on the spatial and temporal availability of food items (Evans 1983), we assessed trophic position of Harris mud crab by investigating variability in stable isotopes ratios ($^{13}\text{C}:^{12}\text{C}$ and $^{15}\text{N}:^{14}\text{N}$, expressed as $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) (Peterson and Fry 1987). Earlier studies (e.g. Nordström et al. 2009, 2010; Karlson et al. 2015) demonstrated spatial and temporal differences in food web structure between various shallow coastal habitats in the northern Baltic Sea. Therefore, we focused our work on two habitats that differed with respect to wave exposure and substrate. To accommodate potential temporal differences during the productive season, we conducted field sampling in both early and late summer. We hypothesized that the two populations of *R. harrisii* would experience somewhat different food availability, which could reveal the plasticity of the species in their food web positioning. In accordance with present knowledge about the species' use of resources, we hypothesised that *R. harrisii* individuals would actively choose prey and favour a structurally more complex and potentially more sheltering habitat over bare substrate.

Methods

Trophic positioning through stable isotope analysis

To assess trophic position, we collected *Rhithropanopeus harrisii* individuals, fish, benthic infauna and epifauna, vascular plants, algae, and detritus from two localities in the Archipelago Sea in June and August 2012. Sampling sites were located 30 km apart in the inner archipelago zone, with a salinity of 5–6 and depth of 1–2 m. The two sites differed markedly in terms of wind and wave exposure as well as the composition of the bottom substrate. Sampling station 1 (Tuorla: 60°40'77"N; 22°44'29"E) was situated in a narrow and shallow strait in the inner archipelago, where vegetation along the shores were dominated by the European common reed *Phragmites australis*. The bottom consisted of mud, which was covered by decaying reeds, and the sediment was sometimes hypoxic under the overlying vegetation. Sampling station 2 (Lempisaari: 60°49'04"N; 21°77'90"E) was situated further out in the archipelago, still surrounded by islands but leaving it more exposed to wind and wave action. Perennial brown algae (*Fucus vesiculosus*) and common reed dominated

the vegetation in the area. The bottom consisted of mud and clay, covered by a layer of loose drifting *F. vesiculosus*.

Sampling of *R. harrisii* was accomplished using wire traps filled with shells or pieces of clay pots that allowed crabs to move in and out freely (Fowler et al. 2013). Some of the crabs at station 2 were collected by hand from drifting stands of *F. vesiculosus*. Fish were collected from some of the crab traps, and additional fish samples were collected with a wire fish-trap (20 mm mesh size). Epi- and in-faunal benthic invertebrates were sampled using an Ekman-Birge grab and push-nets. Plant material and algae were sampled with push-nets and rakes. Thirty two taxa (plants, invertebrates, and fish) were sampled in this study: 26 taxa from station 1 and 24 taxa at station 2, with 18 taxa in common (Table 1).

All samples were frozen as soon as possible and stored at -20 °C for subsequent analyses. In the laboratory, crabs were measured (carapace width, CW in mm), and the sex was determined if possible. The smallest crab for which we could determine to sex was 7 mm CW. However, sex was not determined for many crabs collected in June (station 1: 40 individuals; station 2: 50 individuals). These crabs were mostly 4–10 mm CW.

Depending on the size of the crab, claw muscle tissue, whole claws, or the whole individual was used for analysis of carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) stable isotopes. Muscle tissue was used from fish and the Baltic prawn *Palaemon adspersus* Rathke, 1837. Specimens of epi- and in-faunal benthic invertebrates were analysed whole, except bivalves and gastropods for which shell-free soft tissues were used. Three to five individuals were sampled from each species. All samples were treated with 1 M HCl to remove carbonates. The animal material was dried at 60 °C for 48 h, crushed to a powder in a mortar, and 1 (\pm 0.2) mg of the sample put into tin capsules for analysis. Plant material was dried, crushed to a powder, and 2–3 mg of the powder was put into tin capsules for analysis. The samples were sent to the Stable Isotope Facility of the University of California, Davis (California, USA) for analysis of carbon and nitrogen stable isotope ratios.

Habitat- and food choice experiments

The individuals of *R. harrisii* used in the laboratory experiments were collected two weeks prior to the experiments and were kept in 30 L aquaria filled with seawater (15 °C, salinity 5.5)

Table 1. List of sampled foodweb components found on Station 1 and Station 2 in 2012. A species/food web component found in only one station is indicated in bold.

Secondary consumers:	Station 1	Station 2
<i>Rhithropanopeus harrisi</i>	x	x
<i>Palaemon adspersus</i>	x	x
<i>Perca fluviatilis</i>	x	x
<i>Gymnocephalus cernuus</i>	x	
<i>Gobius niger</i>	x	
<i>Pomatoschistus minutus</i>	x	x
<i>Pungitus pungitus</i>	x	
<i>Rutilus rutilus</i>	x	
<i>Tinca tinca</i>	x	x
<i>Abramis brama</i>	x	x
<i>Blicca bjoerkna</i>	x	
<i>Scardinius erythrophthalmus</i>	x	
Primary consumers:		
<i>Asellus aquaticus</i>	x	x
<i>Gammarus</i> sp.	x	x
<i>Marenzelleria</i> sp.		x
<i>Hediste diversicolor</i>		x
<i>Macoma balthica</i>	x	x
<i>Mya arenaria</i>		x
<i>Mytilus edulis</i>		x
<i>Theodoxus fluviatilis</i>	x	x
<i>Lymnea stagnalis</i>	x	x
<i>Bithynia tentaculata</i>	x	x
Chironomidae	x	x
Ephemeroptera	x	x
Odonata	x	x
Tricoptera	x	
Primary producers:		
<i>Ceratophyllum demersum</i>	x	x
<i>Myriophyllum</i> sp.	x	
<i>Cladophora glomerata</i>	x	x
<i>Enteromorpha intestinalis</i>	x	
<i>Fucus vesiculosus</i> (apikal and basal)		x
Epiphytes (on <i>F. vesiculosus</i>)		x
Detritus	x	x
Decaying <i>Phragmites australis</i>	x	

and equipped with an aerating system. Pieces of clay-pots were added to the aquaria to provide shelter for the crabs, and the individuals were fed frozen chironomids and clams (*Macoma balthica* (Linnaeus, 1758)) during this time. Individuals were fasted for 24 hours prior to the experiments. Both male and female crabs (mean \pm SE CW: 16.1 ± 0.32 mm; range: 8.4 to 21.4 mm; $n = 60$) were used in the experiments but no distinction was made between the two sexes in experiments.

The habitat- and food-choice experiments were conducted in Y-shaped aquaria, which allows for a combination of two resources to be presented to the individual crab simultaneously

(Bonsdorff and Vahl 1982). We used two experimental aquaria (basal area: 40×16 cm; each arm area: 33×10 cm; water height: 8 cm; water volume: 6 L) to conduct trials. A trial consisted of a combination of either two resources or a resource and a control (three possible combinations), and was repeated 15 times. Experimental trials were randomised between the two aquaria, and new individuals were used every trial to avoid any bias in individual crab behaviour or related to the experimental conditions. The aquaria was scrubbed and carefully rinsed with fresh water between trials to remove all traces of the resources. The placement of a resource (habitat or food) or control (seawater, no substrate) in the end of either of the two “arms” was randomised for each trial. The water temperature was kept at 15 °C, salinity at 5.5, and a flow-through system allowed water to flow from each “arm” to the basal area at a rate of 0.15 L/minute, resulting in 100 % water exchange every 40 minutes.

Prior to the start of the experiment, a crab was placed at the basal area, where it was allowed to acclimatize for 10 minutes. A perforated wall between the base and the “arms” prevented the crab from leaving the basal area, but exposed the individual to the water from the “arms” and thereby the potential odour from the resources. When an experimental trial started, the perforated wall was lifted, and we noted the initial choice of the crab and then documented the position of the crab every five minutes for 30 minutes, providing six observations per trial.

In the habitat choice experiment, crabs could choose between soft sediment (clay) and brown algae (*Fucus vesiculosus*). In the food choice experiment, we used the vegetation-associated amphipod *Gammarus* spp. and the sediment-dwelling polychaete *Hediste diversicolor* O. F. Müller, 1776 as food resources. Both prey species were placed inside a transparent net-bag that allowed the crabs to see and smell the prey without consuming it. The control in both the food- and habitat experiment was seawater, no substrate.

Statistical analyses

As a first step, to evaluate if there were any natural breakpoints in carbon isotope values in relation to size of the crab, we conducted a piecewise linear regression analysis using the package Segmented (Muggeo 2008) in R (R Development Core Team 2014). The results showed that there were two breakpoints, one at 11.53 ± 0.36 mm CW and another at 12.32 ± 0.11 mm CW (mean \pm SE)

Figure 1. Piecewise regression plot of *Rhithropanopeus harrisi* size (x-axis) versus $\delta^{13}\text{C}$ -values (y-axis) of all crabs sampled in June and August 2012. Two breakpoints are identified at around 12 mm carapace width size. Slopes of the lines are 0.2163 (for size up to 11.53 mm), -6.529 (11.53 – 12.32 mm) and -0.00271 (> 12.32 mm).

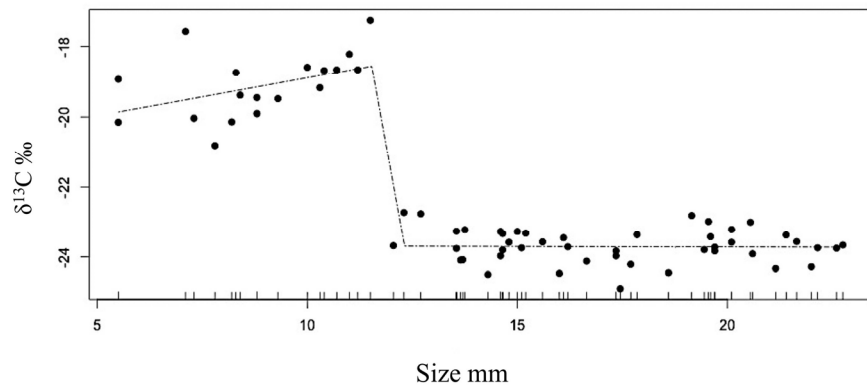


Table 2. Number, sex, and size (carapace width range and mean size, mm) of *Rhithropanopeus harrisi* sampled at Station 1 and Station 2 in June and August 2012.

	June			August		
	n	range	mean ± SE	n	range	mean ± SE
a) Station 1						
Male	17	14.3 – 22.0	18.45 ± 0.60	26	7.45 – 22.75	15.04 ± 0.96
Female	10	13.5 – 16.2	14.97 ± 0.30	15	7 – 13.55	10.32 ± 0.49
Not sexed	40	4.0 – 12.0	7.52 ± 0.28	0		
Total	67			41		
b) Station 2						
Male	6	11.7 – 19.3	16.60 ± 1.16	16	10 – 20.5	15.78 ± 0.87
Female	13	10.0 – 17.5	12.45 ± 0.77	0		
Not sexed	50	4.0 – 10.0	6.70 ± 0.24	8	10 – 15	11.49 ± 0.76
Total	69			24		

(Figure 1). Thus, for all subsequent analysis, we divided crabs into small (< 12 mm CW) and large (> 12 mm CW) size classes.

To assess spatial differences in stable isotope values of small and large size classes of *R. harrisi* and food web components (detritus, primary producers, primary and secondary consumers), separate t-tests were used. If the variances were not homogenous, a t-test with Welch correction was performed.

In order to assess temporal differences in isotope values between the two size classes, we conducted separate Two-way ANOVAs for station 1 and station 2 for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. A comparison of isotopic differences between male and female crabs was analysed with t-tests for the large sized crabs (> 12mm CW) in June only, as not enough data were available for comparison in August.

To analyse the first-choice (resources or control) of crabs in the aquarium experiments, we used Chi-square statistics. One-way ANOVA was used to analyse the choice of crabs made every 5 minutes

(i.e. the position of the crabs in the aquaria), during the 30 min trial. No data transformations were required as the data were normally distributed and the variances homogeneous.

Results

Trophic position and spatial variability

We collected 108 (48 large, 60 small) individuals of *R. harrisi* at station 1 and 93 (26 large and 67 small) at station 2 (Table 2). The largest number of individuals was sampled in June at both stations, and the majority were smaller than 12 mm CW (60 % at station 1 and 84 % at station 2). In August, the small size group accounted for 50 % of the individuals from station 1 and 38 % of those from station 2. The sex ratio was skewed toward more males at both sampling sites. However, in June, sex was not determined for most crabs due the small individual sizes of the animals. Five ovigerous females (38% of all female crabs, 10.7–13.6 mm CW) were obtained from

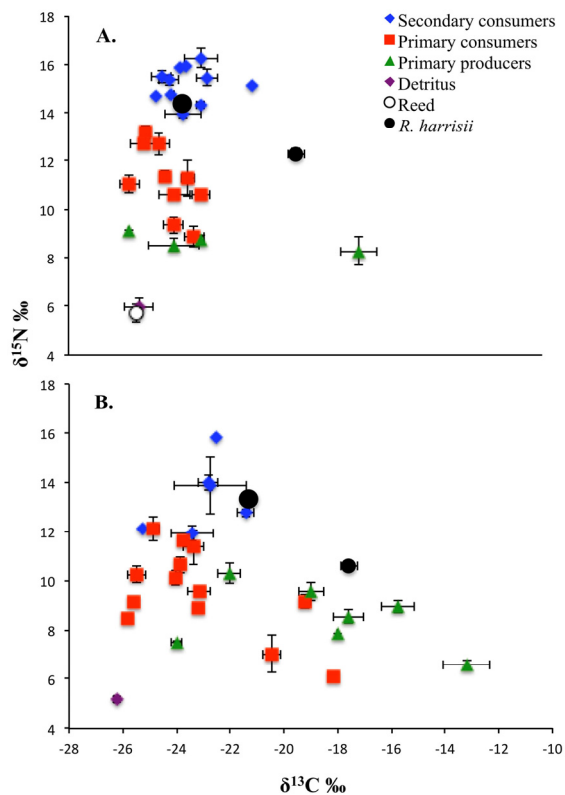


Figure 2. Isotope values ($\delta^{13}\text{C}$ & $\delta^{15}\text{N}$) for organisms sampled in June and August 2012, at A. Station 1 (Tuorla) and B. Station 2 (Lempisaari). Large (> 12 mm) and small (< 12 mm) *Rhithropanopeus harrisii* are indicated by larger and smaller symbol (\bullet), respectively. Secondary consumers: *Abramis brama*¹, *Blicca bjoerkna*¹, *Gobius niger*, *Gymnocephalus cernuus*¹, *Palaemon adspersus*, *Perca fluviatilis*, *Pomatoschistus minutus*, *Pungitius pungitius*¹, *Rutilus rutilus*, *Scardinius erythrophthalmus*¹, *Tinca tinca*. Primary consumers: *Asellus aquaticus*, *Bithynia tentaculata*, Chironomidae, Ephemeroptera, *Gammarus* sp, *Lymnea stagnalis*, *Macoma balthica*, *Marenzelleria* sp², *Mya arenaria*², *Mytilus edulis*², *Hediste diversicolor*², Odonata, *Theodoxus fluviatilis*, Tricoptera¹. Primary producers: *Ceratophyllum demersum*, *Cladophora glomerata*, *Enteromorpha intestinalis*¹, Epiphytes (on Fucus)², *Fucus vesiculosus* (apical)², *F. vesiculosus* (basal)², *Myriophyllum* sp. ¹= species were found on station 1 only, ² = species were found on station 2 only.

station 1 in August, while only males were found at station 2 at that time.

The stable isotope values (June and August combined) of *R. harrisii* at station 1 varied between -24.90 and -17.58 ‰ for $\delta^{13}\text{C}$ and between 10.59 and 15.47 ‰ for $\delta^{15}\text{N}$ (Figure 2A). Crab individuals at station 2 were enriched

in carbon compared to the other station (-22.69 and -15.19 ‰ for $\delta^{13}\text{C}$) and depleted in nitrogen (between 9.07 and 14.31 ‰ for $\delta^{15}\text{N}$) (Figure 2B). This pattern was confirmed for both the large and small size class of crabs when we compared mean values of $\delta^{13}\text{C}$ of *R. harrisii* between stations (large: $t = 13.60$, $df = 31$, $p < 0.0001$, and small: $t = 3.646$, $df = 35$, $p = 0.0009$). Similarly, both size classes differed significantly in terms of $\delta^{15}\text{N}$ between stations (large: $t = 5.733$, $df = 63$, $p < 0.0001$, and small: $t = 4.383$, $df = 38$, $p < 0.0001$).

The same spatial variability in carbon and nitrogen signatures was also found within the other food web components except detritus, which did not vary between stations (Figure 2). At station 1, the values for all sampled food web components ranged between -15.4 and -27.7 for $\delta^{13}\text{C}$ and between 3.4 and 17.3 for $\delta^{15}\text{N}$. At station 2, the corresponding values were -10.7 to -26.5 for $\delta^{13}\text{C}$ and 3.6 to 15.8 for $\delta^{15}\text{N}$. Both secondary and primary consumers were significantly enriched in $\delta^{13}\text{C}$ at station 2 compared to station 1 (secondary consumers: $t = 3.251$, $df = 51$, $p = 0.0020$, primary consumers: $t = 6.099$, $df = 64$, $p < 0.0001$). Carbon stable isotope values of primary producers did not differ significantly between sites. Similarly, a significantly higher $\delta^{15}\text{N}$ value was found at station 1 for secondary ($t = 7.207$, $df = 51$, $p < 0.0001$) and primary consumers ($t = 5.800$, $df = 65$, $p < 0.0001$), as well as primary producers ($t = 3.994$, $df = 9$, $p = 0.0031$).

In terms of the spatial variability in trophic position of the two size classes, the same pattern was seen at both stations. The large size class of *R. harrisii* was located at the same trophic level as fish and other secondary consumers, while small individuals were positioned together with primary consumers, in this case invertebrates (Figure 2). This result supports the clear evidence of an ontogenetic diet change, as initially suggested in the piece-wise linear regression (Figure 1).

Temporal variability of isotope signatures

Carbon and nitrogen stable isotopes of the two size-classes varied over time but the general difference between large and small crabs were constant at both stations. At station 1, a significant interaction between crab size and month was detected for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Table 3). The average $\delta^{13}\text{C}$ value at station 1 in June was 4 ‰ higher in large crabs than small ones (Figure 3), i.e., large crabs were more enriched in carbon. In August, the difference was almost 5 ‰ between

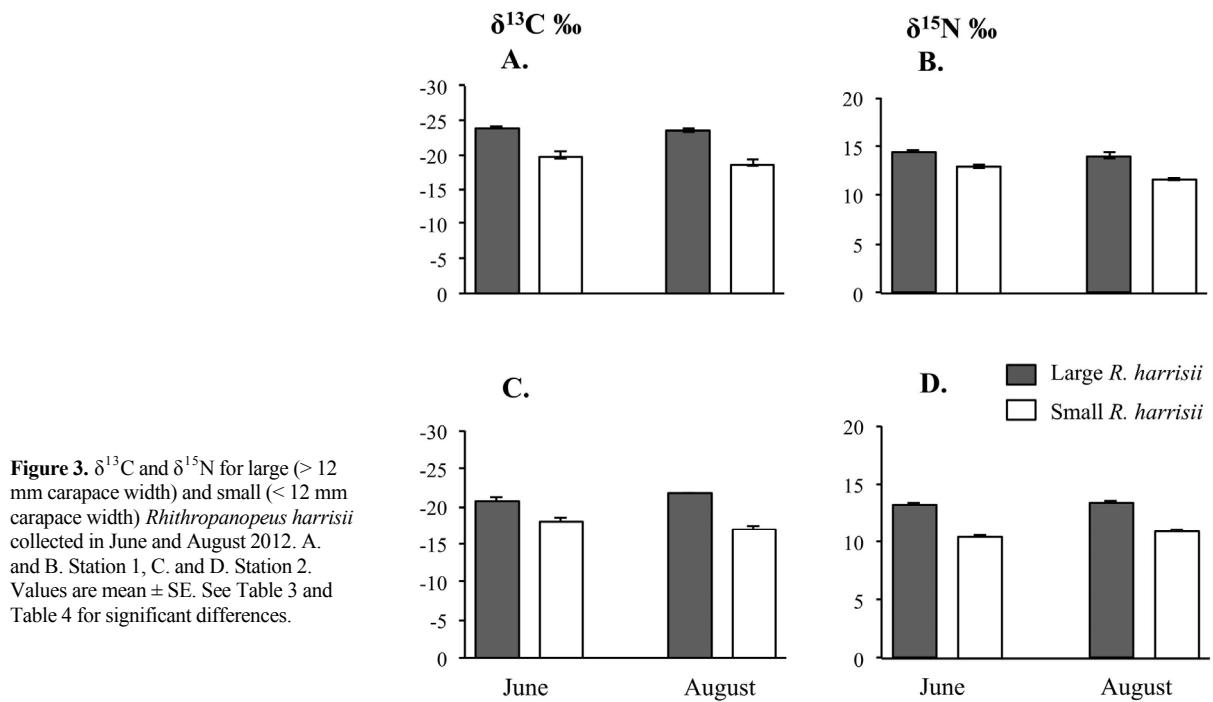


Table 3. Two-factor ANOVA results of a) $\delta^{13}\text{C}$ and b) $\delta^{15}\text{N}$ in *Rhithropanopeus harrisii* of different sizes (small, large) and sampled in different months (June, August) at Station 1. Significant differences are shown in bold.

Treatment	df	MS	F	p
a) $\delta^{13}\text{C}$				
Size	1	248.5	831.0	<0.0001
Month	1	6.279	21.00	<0.0001
Size \times Month	1	2.600	8.694	0.0046
Error	57	0.2990		
b) $\delta^{15}\text{N}$				
Size	1	52.58	119.6	<0.0001
Month	1	10.36	23.56	<0.0001
Size \times Month	1	3.041	6.918	0.0110
Error	57	0.4396		

Table 4. Two-factor ANOVA results of a) $\delta^{13}\text{C}$ and b) $\delta^{15}\text{N}$ in *Rhithropanopeus harrisii* of different sizes (small, large) and sampled in different months (June, August) at Station 2. Significant differences are shown in bold.

Treatment	df	MS	F	p
a) $\delta^{13}\text{C}$				
Size	1	159.5	135.0	<0.0001
Month	1	0.9287	0.7862	0.3806
Size \times Month	1	8.387	7.100	0.0111
Error	40	1.181		
b) $\delta^{15}\text{N}$				
Size	1	64.80	88.79	<0.0001
Month	1	0.1559	0.2136	0.6464
Size \times Month	1	0.02149	0.02945	0.8646
Error	40	0.7298		

the size categories at the same station. The mean $\delta^{15}\text{N}$ was 1.57 ‰ higher in large sized crabs compared to small ones in June and 2.57 ‰ higher in August (Figure 3). The $\delta^{13}\text{C}$ values of large (> 12 mm) crabs did not differ significantly ($p = 0.7630$) between males and females in June, whereas $\delta^{15}\text{N}$ values were significantly higher in females than in males ($t = 2.563$, $df = 25$, $p = 0.0154$).

A slightly different pattern was observed at station 2, where a significant interaction between crab size and month was detected for $\delta^{13}\text{C}$ but not for $\delta^{15}\text{N}$ (Table 4). In parallel to station 1, large crabs were more enriched in carbon compared to small ones and this increased over time (average value of $\delta^{13}\text{C}$ in June was 3 ‰ higher for the larger size class and increased to 4.8 ‰ in August) (Figure 3). Concerning $\delta^{15}\text{N}$, values differed significantly between the two size classes but not over time (between June and August). The $\delta^{15}\text{N}$ values were about 2.5 ‰ higher in large sized crabs compared to the small ones in both months (Figure 3). The isotope values of large crabs did not differ significantly between the sexes ($\delta^{13}\text{C}$ $p = 0.1017$; $\delta^{15}\text{N}$ $p = 0.1312$).

Habitat and food choices

The laboratory experiments showed that *Rhithropanopeus harrisii* actively choose both habitat and prey. There was no significant difference in the initial choice of substrate, whether the crabs were presented with *Fucus vesiculosus* and control ($p = 0.80$), clay and control ($p = 0.80$), or between the two substrates ($p = 0.45$). Over the 30-minute period, however, individuals preferred *F. vesiculosus* to both control and clay (One-way ANOVAs: $F_{1,28} = 32.02$, $p < 0.0001$ and $F_{1,28} = 3.85$, $p = 0.01$, respectively). No significant difference ($p > 0.05$) was found between clay and control.

The initial food choice of *R. harrisii* varied depending on the prey species presented. Crab individuals chose the worm *H. diversicolor* over the control with seawater ($\chi^2 = 9.320$, $df = 1$, $p = 0.0023$), but showed no significant preference between *Gammarus* sp. and control ($p = 0.44$), or between *H. diversicolor* and *Gammarus* sp ($p = 0.80$). When observing the crabs for 30 minutes, however, the individuals spent significantly more time in the area of the aquaria with a food source than in the area with a control (*Gammarus* sp.: One-way ANOVA. $F_{1,28} = 4.31$, $p = 0.05$; *H. diversicolor*: $F_{1,28} = 11.48$, $p = 0.0001$). Similarly to the results of the initial choices, no clear preference of *R. harrisii* between *H. diversicolor* and *Gammarus* sp. was observed over time.

Discussion

We determined the trophic position of Harris mud crab using stable isotope ratios, and identified consistent size-specific differences over time and space. The presence of an ontogenetic diet shift was confirmed through the combination of: i) a natural breakpoint in carbon stable isotopes identified at the size of 12 mm CW; and ii) the positioning of large *Rhithropanopeus harrisii* among the secondary consumers and small individuals with primary consumers. The generalist nature of *R. harrisii* described by the stable isotope ratios was supported by an active food choice but no specific preference for the prey species presented in laboratory experiments. Our hypothesis about substrate preferences of *R. harrisii* was confirmed experimentally by the choice of the structurally more complex bladderack, *Fucus vesiculosus*, over bare mud.

The trophic role(s) resulting from ontogenetic diet shift

Depending on the diet, species may contribute to different trophic pathways and thus, express different trophic roles in an ecosystem (Blondel 2003). Moreover, ontogenetic diet shifts can result in differing juvenile and adult resource use and thus their ecological roles (Werner and Gilliam 1984). Ontogenetic changes in diet are a very common occurrence among invertebrates and fishes (Werner and Gilliam 1984) and are a means to decrease intraspecific competition for food (Aarnio et al. 1998; Arim et al. 2010; Toscano and Griffen 2012). The natural breakpoint in carbon stable isotopes of Harris mud crab at 12 mm CW suggested that large and small *R. harrisii* used different carbon sources and thus there was an ontogenetic diet shift. The analysis of *R. harrisii* stable isotope ratios in bivariate δ -space further supported this observation with small individuals acting as primary consumers while large crabs grouped with fish and other secondary consumers. The enriched $\delta^{13}\text{C}$ values of the individuals in the small size class showed that these had predominately fed on plant material, such as detritus, algae and/or macrophytes. Large crabs appeared to have fed mainly upon invertebrate fauna, which had a carbon signature about 2 ‰ lower than the signature of the crabs themselves.

Previous studies have classified the Harris mud crab as an omnivore feeding on detritus and a large variety of macrofaunal species (Turoboyski 1973). Studies based on stomach contents have had the same conclusion (Czerniejewski and Rybczyk

2008; Hegele-Drywa and Normant 2009). However, previous studies either did not take size into account (Czerniejewski and Rybczyk 2008) or focussed on crabs > 12 mm CW (Hegele-Drywa and Normant 2009). While the species *per se* can be classed as an omnivore (Pimm and Lawton 1978), our findings indicate this to be an over-simplification. The demonstrated ontogenetic switch between feeding as a primary consumer to feeding as a secondary consumer illustrates two distinct trophic roles of *R. harrisii*. In addition, ovigerous females as small as 8.4 mm CW can be found (Fowler et al. 2013; this study), which would mean that the smaller size class (<12 mm CW) includes reproductive individuals and thus is not strictly comprised of juveniles. This makes the ontogenetic diet change and the role as primary consumer interesting because it cannot be explained solely as behaviour linked to the juvenile stage.

The clear difference in trophic position with size is perhaps not surprising in the light of general invertebrate and crab ecology (Werner and Gilliam 1984; Boudreau and Worm 2012). For the invasive crab *Hemigrapsus sanguineus* (De Haan, 1835), Griffen et al. (2012) showed that herbivory decreased with increasing size for females. The shift to more animal tissue in the diet with an increase in crab age/size was assumed to reflect changes in size-specific nutritional requirements (related to molting and growth), and reflected the greater capability of larger individuals to find and handle animal prey (Griffen et al. 2012). These are also possible mechanisms for the observed shift in *R. harrisii* in this study, as are the potential need for an animal-based food source to facilitate energy need for reproduction (Kang and Poulet 2000). Nevertheless, with the existence of mature individuals < 12 mm CW, reproduction clearly occurs on a diet of detritus and other plant material. The lower consumption of animal prey by small crabs could potentially also be linked to differences in habitat usage, and thus food availability, by small and large crabs because of intra-specific competition or predation (Dittel et al. 1995; Nurkse et al. 2015).

When interpreting stable isotope signatures, nature of the tissue analysed needs to be taken into account especially for nitrogen stable isotope analysis. The lower $\delta^{15}\text{N}$ values in the small size class could be partly due to small crabs being analysed as whole organisms while only soft claw tissues were analysed for large crabs. This methodological discrepancy was inevitable because of the small amount of soft tissue material in small crabs. Søreide and Nygård (2012) showed

in their study that $\delta^{15}\text{N}$ values in arctic amphipods were significantly lower in the exoskeleton than in soft tissue, and whole organism signatures were in between the two. Also, $\delta^{15}\text{N}$ values of the whole animals were 1–2 ‰ lower than those of soft tissue, while $\delta^{13}\text{C}$ values were not significantly different between whole-animal and exoskeleton samples.

Spatial and temporal consistency in trophic position

Variability in isotopic signatures in space and time is common. In this study, the trophic structure of the assemblages sampled and the position of both size-classes of *Rhithropanopeus harrisii* were consistent in space (i.e. between two different locations, about 30 km apart). Although $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values differed significantly between the two stations studied, both regarding the whole consumer assemblage and *R. harrisii* in particular, we perceived these as resulting from local nitrogen and carbon dynamics, rather than changes in trophic level. A similar pattern in spatial variability was identified by Nordström et al. (2010) in a study of nine shallow sandy sediment food webs in the northern Baltic Sea where the basic food web components remained the same but the trophic positioning of each varied depending on the local conditions and the local species composition. Furthermore, slight differences in trophic structure and/or positioning of individual species do not necessarily indicate altered or different functional properties of the systems (Törnroos et al. 2013). Moreover, influx of freshwater can affect the natural abundances of $\delta^{15}\text{N}$ in plant tissue as allochthonous nitrogen differs in isotopic signature from autochthonous nitrogen (Peterson and Fry 1987; McClelland and Valiela 1998), while enrichment of $\delta^{15}\text{N}$ in primary producers is known to indicate denitrification, e.g., during hypoxia (Fourqurean et al. 1997). These mechanisms may explain the higher $\delta^{15}\text{N}$ values at station 1, which is a sheltered narrow strait in the inner archipelago close to the coast with a higher volume of fresh water runoff. Additionally, we noted that the sediment at station 1 occasionally experienced hypoxic conditions during the field sampling.

To be able to extrapolate results from stable isotope analysis, it is also important to assess temporal variability in isotope signatures (Akin and Winemiller 2006; Nordström et al. 2009). In this study, we were interested in the intra-seasonal variation in the trophic role of Harris mud crab, and the consistent temporal variability in signatures of large and small *R. harrisii* over the production

period (June to August) strengthened the size-specific difference in trophic position. Temporal fluctuations in stable isotope signatures is common as primary producers often vary in isotope values several times per year (Rolff 2000), which will after a potential lag time be reflected in higher trophic levels (Goering et al. 1990; Nordström et al. 2009). Our results show that the pattern was stable over time although the difference in $\delta^{13}\text{C}$ between small and large crabs increased from June to August at both locations, possibly due to changing isotope values at the base of the food web or changes in food sources, or both (Nordström et al. 2009).

The implication of an active habitat and food choice

Investigating both habitat and feeding choices is important for disentangling invasion impacts and success (Marchetti et al. 2004). As discussed above, trophic patterns may reflect differences in habitat occupancy linked to local food availability and community interactions (Dittel 1995; Nurkse et al. 2015). The results from the laboratory experiments showed that *R. harrisii* preferred the brown alga *Fucus vesiculosus* as habitat over clay or the control (no substrate), and that the crabs were strongly attracted to the odour of both prey organisms offered as food. Crabs in general have well-developed olfactory senses (Weissburg and Zimmer-Faust 1993), and *R. harrisii* uses this trait in search for both suitable habitat and food (Kidawa et al. 2004). Surprisingly, there was no difference between the choice of clay and bare substrate as alternative habitats, although clay naturally had a stronger odour than plain seawater. The burying behaviour of Harris mud crab is well known (Petersen 2006; Fowler et al. 2013) and females in particular need access to suitable sediments when they are about to lay their eggs (Turoboyski 1973). During other periods, crabs are observed to search for shelter under shells, stones, or in vegetation (Ryan 1956; Fowler et al. 2013). We used both male and female individuals in our experiments, and both were primarily attracted to the habitat that provided overhead shelter over clay and the structure-free control. These findings are consistent with other experiments (Nurkse et al. 2015) in which *R. harrisii* chose habitats with boulders covered with *F. vesiculosus* over all other habitats provided (*Cladophora*, unvegetated boulder, silty sand).

In the food choice experiment, both prey species were chosen over the control, but no preference was found between the offered prey types. Our experimental results support previous

findings that the species chooses prey according to availability (Czerniejewski and Rybczyk 2008; Hegele-Drywa and Normant 2009). The preferred first choice was the polychaete *Hediste diversicolor*, however, no statistical differences in overall choice were found. It is possible that *H. diversicolor* left a stronger chemical cue in the water than the amphipods (Velez et al. 2007) and was thus detected more easily by the crabs. In line with studies of olfactory senses in other crab species (Rittschof 1992; Keller et al. 2003) and crustaceans (Weissburg and Zimmer-Faust 1993; Lee and Meyers 2006), the strength of a food signal may be a significant factor modifying food-search behaviour of *R. harrisii*. Kidawa et al. (2004) demonstrated that *R. harrisii* uses the stimulus concentration as a source of information about their distance to a food item. Furthermore, avoidance behaviour of weak food signals may decrease the risk of encountering predators or cannibalistic conspecifics (Kidawa et al. 2004; Tran 2014). Additionally, crabs exposed to food odour repeatedly may lose their motivation to locate the food if they cannot find its source and begin feeding (Ristvey and Rebach 1999; Kidawa et al. 2004). The crabs in our experiments may have lost interest in the prey because they could not reach it through the net-bags, which could explain why there was no difference between choices of prey species over the 30 min period.

Understanding of the trophic and functional roles of non-indigenous species

Recent studies on the role of non-indigenous species in the marine environment underlines the importance of knowing their biology and ecology as a basis for legislation and marine management (Lehtiniemi et al. 2015; Ojaveer et al. 2015), as well as impact prediction (Kolar and Lodge 2001; Dick et al. 2014). We therefore gathered the current biological and ecological trait knowledge of this species worldwide (Table 5) and qualitatively compared a number of traits with those of the native macrofaunal community (from Valanko et al. 2010; Villnäs et al. 2011; Törnroos and Bonsdorff 2012; Törnroos et al. 2015). Törnroos and Bonsdorff (2012) described 12 trait categories expressed by the macrofaunal community along the entire Finnish coastline, i.e., represented the typical functional characteristics of the northern Baltic Sea benthic species. *Rhithropanopeus harrisii* shares eight of these traits with this “type organism” because *R. harrisii* is: of medium size (1–3 cm); reproduces sexually and has separate sexes; is mobile; and

Table 5. Compilation of traits and ecologically important information of *Rhithropanopeus harrisii*.

Trait	Value/category	Reference
Size	Introduced areas: Max*: ♂ 22.8 mm, ♀ 20.2 mm Range: ♂ 1.8-22.9, ♀ 1.8-20.2 mm Native areas: ♂ 4.1–14.6 mm, ♀ 4.4–12.6 mm	*This study Fowler et al. 2013, Turoboyski 1973 Ryan 1956
Size of maturity	Introduced areas: 8.4mm*, 4–5mm Native areas: ♂ 4-5 mm, ♀ 4.4–5.5 mm	*This study, Turoboyski 1973, Williams 1984 Ryan 1956
Time to maturity	0.5 yr.	Williams 1984
Reproductive type	Sexual repr. (Gonochoristic)	Derived
Developmental type	Ovoviviparous	Derived
Larval type	Lecitotrophic (actively feeding)	Williams 1984
Larval duration	16 days (~ 30d in fully marine areas), 4 stages	Williams 1984
Larval migration	Vertical migration	Petersen 2006
Reproductive season	June – August (temp. dependent)	Fowler et al. 2013, *This study
Egg production	1200–4800 eggs (environmental-dependent)	Turoboyski 1973, *This study
*Trophic level	Primary consumers: ind. < 12mm Secondary consumers: ind. > 12mm	*This study
*Stable isotope values	Average(sd) $\Delta^{13}\text{C}$: -18.4 \pm 1.64 (ind. < 12mm) -22.8 \pm 1.4 (ind. > 12mm) Average(sd) $\Delta^{15}\text{N}$: 11.4 \pm 1.2 (ind. < 12mm) -14.0 \pm 0.8 (ind. > 12mm)	*This study *This study
*Diet (include ontogenetic shift)	Polychaetes (e.g. <i>Hediste</i> sp.) Crustaceans (e.g. <i>Gammarus</i> spp.) Molluscs (e.g. <i>Macoma balthica</i>) Detritus	*This study, Turoboyski 1973, Czerniejewski and Rybczyk 2008, Hegele-Drywa and Normant 2009
Resource capture method	Maxillipeds, maxillae, mandibles (adult), suspension feeder (larvae)	Derived, Williams 1984
Living habit	Epibenthic, Infauna	Derived
Mobility	Mobile	Derived
Movement type	Crawler, Burrower	Derived, *This study
Migration	Potentially to deeper waters in winter	Turoboyski 1973
Ecological information		
Salinity tolerance	0.5 – >20 psu 2.5 – 40 psu (larvae lab)	Boyle et al. 2010, Ryan 1956 Costlow et al. 1966
Temperature tolerance	15 – 35 °C (larvae lab) at least +4 °C (adult)	Costlow et al. 1966 Derived from Finnish water temperatures in winter
Depth range	0–20m (max 37m)	Hegele-Drywa and Normant 2014,
Habitat preferences	Various: e.g. debris on seafloor, canopy-forming algae such as bladder-rack, mud, gravel, oyster and mussel reefs.	*This study, Fowler et al. 2013

lives in or on the top 1 cm of the sediment; and smaller crabs consume both detritus and plant material (Table 5). Furthermore, another trait that was found among the typical benthic species characteristics was diffusive mixing as a bioturbation mode (Törnroos and Bonsdorff 2012). While bioturbation by Harris mud crab at the sediment-water interface has not been investigated directly, the normal movements and feeding on the surface (Turoboyski 1973; Nurkse et al. 2015) most certainly moves sediments around (Kristensen et al. 2012). On the other hand, the burrowing

behavior of *R. harrisii* (Turoboyski 1973; Peterson 2006; Fowler et al. 2013) may represent more elaborate bioturbation effect similar to regenerator or gallery-diffusers that aid bio-irrigation (Kristensen et al. 2012) such as fiddler crabs (Huang et al. 2007). *R. harrisii* matures in about half a year in the Baltic Sea (Turoboyski 1973) and has lecithotrophic larval development (Table 5), which are some of the less common characteristics of the northern Baltic Sea macrofauna (Valanko et al. 2010; Törnroos and Bonsdorff 2012), and may in part explain its success in its new environment.

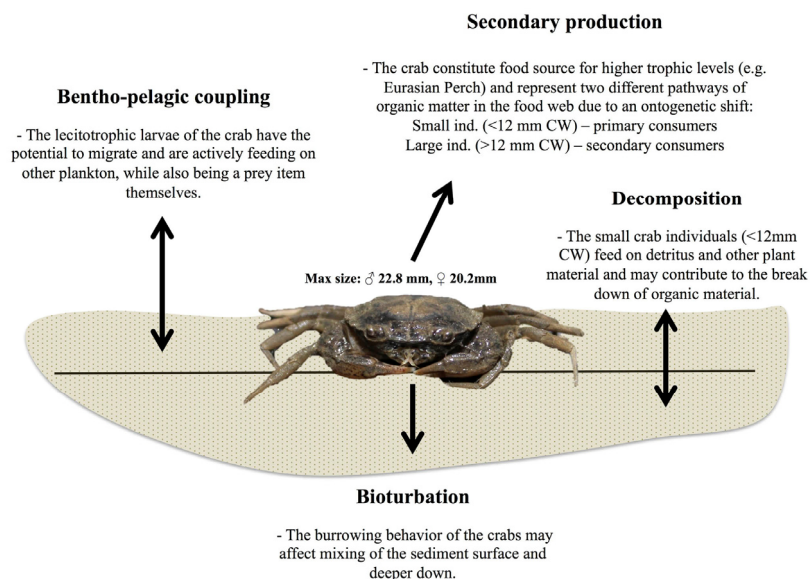


Figure 4. Conceptual figure showing ecosystem functions, to which the non-indigenous *Rhithropanopeus harrisii* potentially could contribute, based on its species-specific traits.

Sharing these traits with the natural macrofaunal community does not mean that the effect of *R. harrisii* on the functioning of the benthic ecosystem could not substantially differ from other benthic species (e.g. in terms of rates). However, it does suggest that the Harris mud crab qualitatively adds to the already present functional (trait) diversity, rather than bringing in something completely new. Disentangling this type of trait information further, in relation to ecosystem functions (Symstad et al. 2003; Violle et al. 2007; Reiss et al. 2009) will help broaden our understanding of the many roles of species in ecosystems (Hooper et al. 2005). Using the species-specific traits (Table 5), it is possible to conceptually illustrate the ecosystem functions of the non-indigenous *R. harrisii* (Figure 4). It is consumed by common predators, e.g., Eurasian perch (*Perca fluviatilis* Linnaeus, 1758), pikeperch (*Sander lucioperca* (Linnaeus, 1758)), and fourhorned sculpin (*Myoxocephalus quadricornis* (Linnaeus, 1758)) (Fowler et al. 2013), and thus contributes to secondary production. It has a role in bioturbation of the surface layer of the sediment. Due to its lecithotrophic larval mode, it would be involved in benthic-pelagic coupling as larvae both consume other zooplankton and are prey for both benthic and pelagic organisms (Marcus and Boero 1998; Raffaelli et al. 2003). Finally, the consumption of detritus and other plant material results in a breakdown and excretion of organic matter, which subsequently is available

to the meiofaunal and bacterial community. Whether the net effect of *R. harrisii* on these ecosystem functions is positive and/or negative, remains to be investigated. Non-indigenous species can affect ecological processes positively and negatively (Thomsen et al. 2015). For example, modelling work (Norkko et al. 2012) suggests the introduced spionid polychaete *Marenzelleria* spp. may enhance phosphorus retention in sediments and thus reduce phosphorus release from bottom waters. This could be interpreted as positive driver of change influencing the function of the system (Norkko et al. 2012; Kauppi et al. 2015). Previously work on *R. harrisii* have stressed potential negative effects, such as the species possibility to displace native organisms (Zaitsev and Öztürk 2001; Fowler et al. 2013; Nurkse et al. 2015). As demonstrated in this study, *R. harrisii* may also contribute positively to the ecosystem functioning, especially in a species-poor system such as the Baltic Sea.

Acknowledgements

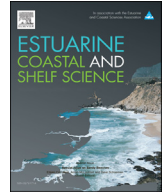
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References

- Aarnio K, Bonsdorff E, Rosenback N (1998) Food and feeding habits of juvenile flounder, *Platichthys flesus* (L.), and turbot, *Scophthalmus maximus* L., in the Åland Archipelago, northern Baltic Sea. *Journal of Sea Research* 36: 311–320
- Akin S, Winemiller KO (2006) Seasonal variation in food web composition and structure in a temperate tidal estuary. *Estuaries and Coasts* 29(4): 552–567, <http://dx.doi.org/10.1007/BF02784282>
- Anger K (1991) Effects of temperature and salinity on the larval development of the Chinese mitten crab *Eriocheir sinensis* (Decapoda: Grapsidae). *Marine Ecology Progress Series* 72: 103–110, <http://dx.doi.org/10.3354/meps072103>
- Arim M, Abades SR, Laufer G, Loureiro M, Marquet PA (2010) Food web structure and body size: trophic position and resource acquisition. *Oikos* 119: 147–153, <http://dx.doi.org/10.1111/j.1600-0706.2009.17768.x>
- Blondel J (2003) Guilds or functional groups: does it matter? *Oikos* 100: 223–231, <http://dx.doi.org/10.1034/j.1600-0706.2003.12152.x>
- Bonsdorff E (2006) Zoobenthic diversity-gradients in the Baltic Sea: Continuous post-glacial succession in a stressed ecosystem. *Journal of Experimental Marine Biology and Ecology* 330: 383–391, <http://dx.doi.org/10.1016/j.jembe.2005.12.041>
- Bonsdorff E, Blomqvist EM (1993) Biotic couplings on shallow water soft bottoms – examples from the northern Baltic Sea. *Oceanography and Marine Biology - An Annual Review* 31: 153–176
- Bonsdorff E, Vahl O (1982) Food preference of the sea urchins *Echinus acutus* and *E. esculentus*. *Marine Behavior & Physiology* 8: 243–248, <http://dx.doi.org/10.1080/10236248209387021>
- Boudreau SA, Worm B (2012) Ecological role of large benthic decapods in marine ecosystems: a review. *Marine Ecology Progress Series* 469: 195–213, <http://dx.doi.org/10.3354/meps09862>
- Boyle T Jr, Keith D, Pfau R (2010) Occurrence, reproduction, and population genetics of the estuarine mud crab, *Rhithropanopeus harrisi* (Gould) (Decapoda, Panopidae) in Texas freshwater reservoirs. *Crustaceana* 83(4): 493–505, <http://dx.doi.org/10.1163/001121610X492148>
- Bremner J, Rogers SI, Frid CLJ (2006) Methods for describing ecological functioning of marine benthic assemblages using biological trait analysis (BTA). *Ecological Indicators* 6: 609–622, <http://dx.doi.org/10.1016/j.ecolind.2005.08.026>
- Brockernhoff A, McLay C (2011) Human-mediated spread of alien crabs. In: Galil BS, Clark PF, Carlton JT (eds.), In the Wrong Place – Alien Marine Crustaceans: Distribution, Biology and Impacts. *Invading Nature, Springer Series in Invasion Ecology* 6: 27–106
- Catford JA, Jansson R, Nilsson C (2009) Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. *Diversity and Distributions* 15: 22–40, <http://dx.doi.org/10.1111/j.1472-4642.2008.00521.x>
- Cohen AN, Carlton JT (1995) Biological Study, nonindigenous aquatic species in a United States Estuary: A case study of the biological invasions of the San Francisco Bay and Delta, A Report for the U.S. Fish and Wildlife Service, 246 pp
- Cohen AN, Carlton JT (1998) Accelerating invasion rate in a highly invaded estuary. *Science* 279: 555–557, <http://dx.doi.org/10.1126/science.279.5350.555>
- Costlow JD Jr, Bookhout CG, Monroe RJ (1966) Studies on the larval development of the crab *Rhithropanopeus harrisi* (Gould). I Effect of salinity and temperature on larval development. *Physiological Zoology* 35: 79–93
- Czerniejewski P, Rybczyk A (2008) Body weight, morphometry, and diet of the mud crab, *Rhithropanopeus harrisi tridentatus* (Maitland, 1874) in the Odra estuary, Poland. *Crustaceana* 81: 1289–1299, <http://dx.doi.org/10.1163/156854008X369483>
- David M, Gollasch S, Leppäkoski E (2013) Risk assessment for exemptions from ballast water management – the Baltic Sea case study. *Marine Pollution Bulletin* 75: 205–217, <http://dx.doi.org/10.1016/j.marpolbul.2013.07.031>
- de Juan S, Thrush SF, Demestre M (2007) Functional changes as indicators of trawling disturbance on a benthic community located in a fishing ground (NW Mediterranean Sea). *Marine Ecology Progress Series* 334: 117–129, <http://dx.doi.org/10.3354/meps334117>
- Dick JTA, Alexander ME, Jeschke JM, Ricciardi A, MacIsaac HJ, Robinson TB, Kumschick S, Weyl OLF, Dunn AM, Hatcher MJ, Paterson RA, Farnsworth KD, Richardson DM (2014) Advancing impact prediction and hypothesis testing in invasion ecology using a comparative functional response approach. *Biological Invasions* 16: 735–753, <http://dx.doi.org/10.1007/s10530-013-0550-8>
- Dittel AI, Hines AH, Ruiz GM, Ruffin KK (1995) Effects of shallow water refuge on behavior and density-dependent mortality of juvenile blue crabs in Chesapeake Bay. *Bulletin of Marine Science* 57: 902–916
- Dobb JA, Dick JTA, Alexander ME, Macneil C, Dunn AM, Aldridge D (2014) Predicting the ecological impacts of a new freshwater invader: functional responses and prey selectivity of the 'killer shrimp', *Dikerogammarus villosus*, compared to the native *Gammarus pulex*. *Freshwater Biology* 59: 337–352, <http://dx.doi.org/10.1111/fwb.12268>
- Elmgren R, Hill C (1997) Ecosystem Function at Low Biodiversity - The Baltic Example. In: Ormond RFG, Gage J, Angel M (eds), *Marine Biodiversity: Patterns and Processes*. Cambridge University Press Cambridge, pp 319–336, <http://dx.doi.org/10.1017/cbo9780511752360.015>
- Evans S (1983) Production, predation and food niche segregation in a marine shallow soft-bottom community. *Marine Ecology Progress Series* 10: 147–157, <http://dx.doi.org/10.3354/meps010147>
- Forward RB Jr (2009) Larval biology of the crab *Rhithropanopeus harrisi* (Gould): a synthesis. *Biological Bulletin* 216: 243–256
- Fourqurean JW, Moore TO, Fry B, Hollibaugh JT (1997) Spatial and temporal variation in C:N:P ratios, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$ of eelgrass *Zostera marina* as indicators of ecosystem processes, Tomales Bay, California, USA. *Marine Ecology Progress Series* 157: 147–157, <http://dx.doi.org/10.3354/meps157147>
- Fowler AE, Forsström T, von Numers M, Vesakoski O (2013) The North American mud crab *Rhithropanopeus harrisi* (Gould, 1841) in newly colonized Northern Baltic Sea: distribution and ecology. *Aquatic Invasions* 8: 89–96, <http://dx.doi.org/10.3391/ai.2013.8.1.10>
- Goering J, Alexander V, Haubenstock N (1990) Seasonal variability of stable carbon and nitrogen isotope ratios of organisms in a North Pacific bay. *Estuarine, Coastal and Shelf Science* 30: 239–260, [http://dx.doi.org/10.1016/0272-7714\(90\)90050-2](http://dx.doi.org/10.1016/0272-7714(90)90050-2)
- Gollasch S, Leppäkoski E (2007) Risk assessment and management scenarios for ballast water mediated species introductions in the Baltic Sea. *Aquatic Invasions* 2: 313–340, <http://dx.doi.org/10.3391/ai.2007.2.4.3>
- Gothland M, Dauvin JC, Denis L, Dufossé F, Jobert S, Ovaert J, Pezy JP, Tous Rius A, Spilmont N (2014) Biological traits explain the distribution and colonisation ability of the invasive shore crab *Hemigrapsus takanoi*. *Estuarine, Coastal and Shelf Science* 142: 41–49, <http://dx.doi.org/10.1016/j.ecss.2014.03.012>
- Goodenough AE (2010) Are the ecological impacts of alien species misrepresented? A review of the “native good, aliens bad” philosophy. *Community Ecology* 11: 13–21, <http://dx.doi.org/10.1556/ComEc.11.2010.1.3>
- Gribben PE, l'Ons S, Phillips NE, Geange SW, Wright JT, Murray BR (2013) Biogeographic comparisons of the traits and abundance of an invasive crab throughout its native and invasive ranges. *Biological Invasions* 15: 1877–1885, <http://dx.doi.org/10.1007/s10530-013-0416-0>
- Griffen BD, Altman I, Bess BM, Hurley J, Penfield A (2012) The role of foraging in the success of invasive Asian shore crabs in New England. *Biological Invasions* 14: 2545–2558, <http://dx.doi.org/10.1007/s10530-012-0251-8>

- Grosholz ED, Ruiz GM (2003) Biological invasions drive size increases in marine and estuarine invertebrates. *Ecology Letters* 6: 700–705, <http://dx.doi.org/10.1046/j.1461-0248.2003.00495.x>
- Hayes KR, Barry SC (2008) Are there any consistent predictors of invasion success? *Biological Invasions* 10: 483–506, <http://dx.doi.org/10.1007/s10530-007-9146-5>
- Hegele-Drywa J, Normant M (2009) Feeding ecology of the American crab *Rhithropanopeus harrisi* (Crustacea, Decapoda) in the coastal waters of the Baltic Sea. *Oceanologia* 51: 361–375, <http://dx.doi.org/10.5697/oc.51-3.361>
- Hegele-Drywa J, Normant M (2014) Non-native crab *Rhithropanopeus harrisi* (Gould, 1984) – a new component of the benthic communities in the Gulf of Gdansk (southern Baltic Sea). *Oceanologia* 56: 125–139, <http://dx.doi.org/10.5697/oc.56-1.125>
- Hooper DU, Chapin III FS, Ewel JJ, Hector A, Inchausti P, Lavorel S, Lawton JH, Lodge DM, Loreau M, Naeem S, Schmid B, Setälä H, Symstad AJ, Vandermeer J, Wardle DA (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs* 75: 3–35, <http://dx.doi.org/10.1890/04-0922>
- Huang K, Boudreau BP, Reed DC (2007) Simulated fiddler-crab sediment mixing. *Journal of Marine Research* 65: 491–522, <http://dx.doi.org/10.1357/002224007782689120>
- Jeschke JM, Bacher S, Blackburn TM, Dick JTA, Essl F, Evans T, Gaertner M, Hulme PE, Kuhn I, Mrugala A, Pergl J, Pyšek P, Rabitsch W, Riccardi A, Richardson DM, Sendek A, Vilà M, Winter M, Kumschick A (2014) Defining the impact of non-native species. *Conservation Biology* 28: 1188–1194, <http://dx.doi.org/10.1111/cobi.12299>
- Kang HK, Poulet SA (2000) Reproductive success in *Calanus helgolandicus* as a function of diet and egg cannibalism. *Marine Ecology Progress Series* 201: 241–250, <http://dx.doi.org/10.3354/meps201241>
- Karlson AM, Gorokhova E, Elmgren R (2015) Do deposit-feeders compete? Isotopic niche analysis of an invasion in a species-poor system. *Scientific Reports* 5: 9715, <http://dx.doi.org/10.1038/srep09715>
- Kauppi L, Norkko A, Norkko J (2015) Large-scale species invasion into a low-diversity system: spatial and temporal distribution of the invasive polychaetes *Marenzelleria* spp. in the Baltic Sea. *Biological Invasions* 17: 2055–2074, <http://dx.doi.org/10.1007/s10530-015-0860-0>
- Keller TA, Powell I, Weissburg J (2003) Role of olfactory appendages in chemically mediated orientation of blue crabs. *Marine Ecology Progress Series* 261: 217–231, <http://dx.doi.org/10.3354/meps261217>
- Kidawa A, Markowska M, Rakusa-Suszczewski S (2004) Chemosensory behaviour in the mud crab, *Rhithropanopeus harrisi* *tredentatus* from Martwa Wisla Estuary (Gdansk Bay, Baltic Sea). *Crustaceana* 77: 897–908, <http://dx.doi.org/10.1163/1568540042781711>
- Kolar CS, Lodge DM (2001) Progress in invasion biology: predicting invaders. *Trends in Ecology & Evolution* 16: 199–204, [http://dx.doi.org/10.1016/S0169-5347\(01\)02101-2](http://dx.doi.org/10.1016/S0169-5347(01)02101-2)
- Kristensen E, Penha-Lopes G, Delefosse M, Valdemarsen T, Quintana CO, Banta GT (2012) What is bioturbation? The need for a precise definition for fauna in aquatic sciences. *Marine Ecology Progress Series* 446: 285–302, <http://dx.doi.org/10.3354/meps09506>
- Lee PG, Meyers SP (2006) Chemoattraction and feeding simulation in crustaceans. *Aquaculture Nutrition* 2: 157–164, <http://dx.doi.org/10.1111/j.1365-2095.1996.tb00055.x>
- Lehtiniemi M, Ojaveer H, David M, Galil B, Gollasch S, McKenzie C, Minchin D, Occhipinti-Ambrogi A, Olenin S, Pederson J (2015) Dose of truth - monitoring marine non-indigenous species to serve legislative requirements. *Marine Policy* 54: 26–35, <http://dx.doi.org/10.1016/j.marpol.2014.12.015>
- Leppäkoski E, Shiganova T, Alexandrov B (2009) European enclosed and semi-enclosed seas. In: Rilov G, Crooks J (eds) *Biological Invasions in Marine Ecosystems: Ecological, Management, and Geographic Perspectives. Ecological Studies* 204: 529–547, http://dx.doi.org/10.1007/978-3-540-79236-9_30
- Leppäranta M, Myrberg K (2009) Physical oceanography of the Baltic Sea. Geophysical Sciences. Springer-Verlag, Berlin, Heidelberg, 378 pp, <http://dx.doi.org/10.1007/978-3-540-79703-6>
- Marchetti MP, Moyle PB, Levine R (2004) Invasive species profiling? Exploring the characteristics of non-native fishes across invasions stages in California. *Freshwater Biology* 49: 646–661, <http://dx.doi.org/10.1111/j.1365-2427.2004.01202.x>
- Marcus NH, Boero F (1998) Minireview: the importance of benthic-pelagic coupling and the forgotten role of life-cycles in coastal aquatic systems. *Limnology and Oceanography* 43: 763–768, <http://dx.doi.org/10.4319/lo.1998.43.5.0763>
- McClelland JW, Valiela I (1998) Linking nitrogen in estuarine producers to land-derived sources. *Limnology and Oceanography* 43: 577–585, <http://dx.doi.org/10.4319/lo.1998.43.4.0577>
- Muggeo VMR (2008) segmented: an R package to fit regression models with broken-line relationships. *R News* 8/1: 20–25
- Nordström M, Aarnio K, Bonsdorff E (2009) Temporal variability of a benthic food web: patterns and processes in a low-diversity system. *Marine Ecology Progress Series* 378: 13–26, <http://dx.doi.org/10.3354/meps07872>
- Nordström MC, Lindblad P, Aarnio K, Bonsdorff E (2010) A neighbour is a neighbour? Consumer diversity, trophic function, and spatial variability in benthic foodwebs. *Journal of Experimental Marine Biology and Ecology* 391: 101–111, <http://dx.doi.org/10.1016/j.jembe.2010.06.015>
- Norkko J, Reed DC, Timmermann K, Norkko A, Gustafsson BG, Bonsdorff E, Slomp CP, Carstensen J, Conley DJ (2012) A welcome can of worms? Hypoxia mitigation by an invasive species. *Global Change Biology* 18: 422–434, <http://dx.doi.org/10.1111/j.1365-2486.2011.02513.x>
- Nurkse K, Kotta J, Orav-Kotta H, Pärmoja M (2015) Laboratory analysis of the habitat occupancy of the crab *Rhithropanopeus harrisi* (Gould) in an invaded ecosystem: the north-eastern Baltic Sea. *Estuarine, Coastal and Shelf Science* 154: 152–157, <http://dx.doi.org/10.1016/j.ecss.2014.12.046>
- Ojaveer H, Galil BS, Campbell ML, Carlton JT, Canning-Clode J, Cook EJ, Davidson AD, Hewitt CL, Jelmer A, Marchini A, McKenzie CH, Minchin D, Occhipinti-Ambrogi A, Olenin S, Ruiz G (2015) Classification of non-indigenous species based on their impacts: Considerations for application in marine management. *PLoS Biology* 13(4): e1002130, <http://dx.doi.org/10.1371/journal.pbio.1002130>
- Ojaveer H, Gollasch S, Jaanus A, Kotta J, Laine AO, Minde A, Normant M, Panov VE (2007) Chinese mitten crab *Eriocheir sinensis* in the Baltic Sea – a supply-side invader? *Biological Invasions* 9: 409–418, <http://dx.doi.org/10.1007/s10530-006-9047-z>
- Paavola M, Olenin S, Leppäkoski E (2005) Are invasive species most successful in habitats of low native species richness across European brackish water seas? *Estuarine, Coastal and Shelf Science* 64: 738–750, <http://dx.doi.org/10.1016/j.ecss.2005.03.021>
- Parker IM, Simberloff D, Lonsdale WM, Goodell K, Wonham M, Kareiva PM, Williamson MH, Von Holle B, Moyle PB, Byers JE, Goldwasser L (1999) Impact: toward a framework for understanding the ecological effects of invaders. *Biological Invasions* 1: 3–19, <http://dx.doi.org/10.1023/A:1010034312781>
- Paterson RA, Dick JTA, Pritchard DW, Ennid M, Hatcher MJ, Dunn AM (2015) Predicting invasive species impacts: a community module functional response approach reveals context dependencies. *Journal of Animal Ecology* 84: 453–463, <http://dx.doi.org/10.1111/1365-2656.12292>
- Petersen C (2006) Range expansion in the northeast Pacific by an estuary mud crab – a molecular study. *Biological Invasions* 8: 565–576, <http://dx.doi.org/10.1007/s10530-005-0160-1>
- Peterson JP, Fry B (1987) Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* 18: 293–320, <http://dx.doi.org/10.1146/annurev.es.18.110187.001453>
- Pimm SL, Lawton JH (1978) On feeding on more than one trophic level. *Nature* 275: 542–544, <http://dx.doi.org/10.1038/275542a0>

- R Development Core Team (2014) R: A language and environment for statistical computing. The R Foundation for statistical computing, Vienna, Austria 3-900051-07-0 (Available online at <http://www.R-project.org/>)
- Raffaelli D, Bell E, Weithoff G, Matsumoto A, Cruz-Motta JJ, Kershaw P, Parker R, Parry D, Jones M (2003) The ups and downs of benthic ecology: consideration of scale, heterogeneity and surveillance for benthic-pelagic coupling. *Journal of Experimental Marine Biology and Ecology* 285–286: 191–203, [http://dx.doi.org/10.1016/S0022-0981\(02\)00527-0](http://dx.doi.org/10.1016/S0022-0981(02)00527-0)
- Reiss J, Birdle JR, Montoy JM, Woodward G (2009) Emerging horizons in biodiversity and ecosystem functioning research. *Trends in Ecology & Evolution* 24: 505–514, <http://dx.doi.org/10.1016/j.tree.2009.03.018>
- Rittschof D (1992) Chemosensation in the daily life of crabs. *American Zoologist* 32: 363–369, <http://dx.doi.org/10.1093/icb/32.3.363>
- Ristvey A, Rebach S (1999) Enhancement of the response of rock crabs, *Cancer irroratus*, to prey odors following feeding experience. *The Biological Bulletin* 197: 361–367, <http://dx.doi.org/10.2307/1542790>
- Rolff C (2000) Seasonal variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of size-fractionated plankton at a coastal station in the northern Baltic proper. *Marine Ecology Progress Series* 203: 47–65, <http://dx.doi.org/10.3354/meps203047>
- Ryan EP (1956) Observations on the life histories and the distribution of the Xanthidae (mud crabs) of Chesapeake Bay. *American Midland Naturalist* 56: 138–162, <http://dx.doi.org/10.2307/2422450>
- Simberloff D, Martin J-L, Genovesi P, Maris V, Wardle DA, Aronson J, Courchamp F, Galil B, García-Berthou E, Pascal M, Pyšek P, Sousa R, Tabacchi E, Vilà M (2013) Impacts of biological invasions: what's what and the way forward. *Trends in Ecology & Evolution* 28: 58–66, <http://dx.doi.org/10.1016/j.tree.2012.07.013>
- Solan M, Raffaelli DG, Paterson DM, White PCL, Pierce GJ (2006) Marine biodiversity and ecosystem function: empirical approaches and future challenges. Introduction. *Marine Ecology Progress Series* 311: 175–178, <http://dx.doi.org/10.3354/meps311175>
- Stachowicz JJ, Fried H, Osman RW, Whitlatch RB (2002) Biodiversity, invasion resistance, and marine ecosystem function: reconciling pattern and process. *Ecology* 83: 2575–2590, [http://dx.doi.org/10.1890/0012-9658\(2002\)083\[2575:BIRAME\]2.0.CO;2](http://dx.doi.org/10.1890/0012-9658(2002)083[2575:BIRAME]2.0.CO;2)
- Strayer DL (2012) Eight questions about invasions and ecosystem functioning. *Ecology Letters* 15: 1199–1210, <http://dx.doi.org/10.1111/j.1461-0248.2012.01817.x>
- Symstad AJ, Chapin III FS, Wall DH, Gross KL, Huenneke LF, Mittelbach GG, Peters DPC, Tilman D (2003) Long-term and large-scale perspectives on the relationship between biodiversity and ecosystem functioning. *BioScience* 53: 89–98, [http://dx.doi.org/10.1641/0006-3568\(2003\)053\[0089:LTALSP\]2.0.CO;2](http://dx.doi.org/10.1641/0006-3568(2003)053[0089:LTALSP]2.0.CO;2)
- Søreide JE, Nygård H (2012) Challenges using stable isotopes for estimating trophic levels in marine amphipods. *Polar Biology* 35: 447–453, <http://dx.doi.org/10.1007/s00300-011-1073-3>
- Thomsen MS, Wernberg T, Schiel D (2015) Invasions by non-indigenous species. In: Crowe TP, Frid CLJ (eds), *Marine Ecosystems: Human Impacts on Biodiversity, Functioning and Services*. Cambridge University Press, pp 274–334, <http://dx.doi.org/10.1017/cbo9781139794763.010>
- Toscano BJ, Griffen BD (2012) Predatory crab size diversity and bivalve consumption in oyster reefs. *Marine Ecology Progress Series* 445: 65–74, <http://dx.doi.org/10.3354/meps09461>
- Tran MV (2014) The scent of cannibalism: the olfactory basis of cannibalism in hermit crabs. *Journal of Experimental Marine Biology and Ecology* 457: 8–14, <http://dx.doi.org/10.1016/j.jembe.2014.03.019>
- Turoboyski K (1973) Biology and ecology of the crab *Rhithropanopeus harrisii* spp. *tridentatus*. *Marine Biology* 23: 303–313, <http://dx.doi.org/10.1007/BF00389338>
- Törnroos A, Bonsdorff E (2012) Developing the multitrait concept for functional diversity: lessons from a system rich in functions but poor in species. *Ecological Applications* 22: 2221–2236, <http://dx.doi.org/10.1890/11-2042.1>
- Törnroos A, Norström MC, Bonsdorff E (2013) Coastal habitats as surrogates for taxonomic, functional and trophic structures of benthic faunal communities. *PloS ONE* 8(10): e78910, <http://dx.doi.org/10.1371/journal.pone.0078910>
- Törnroos A, Bonsdorff E, Bremner J, Blomqvist M, Josefson AF, Garcia C, Warzocha J (2015) Marine benthic ecological functioning over decreasing taxonomic richness. *Journal of Sea Research* 98: 49–56, <http://dx.doi.org/10.1016/j.seares.2014.04.010>
- Valanko S, Norikko A, Norkko J (2010) Strategies of post-larval dispersal in non-tidal soft-sediment communities. *Journal of Experimental Marine Biology and Ecology* 384: 51–60, <http://dx.doi.org/10.1016/j.jembe.2009.12.012>
- Van Kleunen M, Dawson W, Schlaepfer D, Jeschke JM, Fischer M (2010) Are invaders different? A conceptual framework of comparative approaches for assessing determinants of invasiveness. *Ecology Letters* 13: 947–958, <http://dx.doi.org/10.1111/j.1461-0248.2010.01503.x>
- Velez Z, Hubbard PC, Hardege JD, Barata EN, Canário AVM (2007) The contribution of amino acids to the odour of a prey species in the Senegalese sole (*Solea senegalensis*). *Aquaculture* 265: 336–342, <http://dx.doi.org/10.1016/j.aquaculture.2007.02.029>
- Villéger S, Miranda JR, Hernández DF, Mouillot D (2010) Contrasting changes in taxonomic vs. functional diversity of tropical fish community after habitat degradation. *Ecological Applications* 20: 1512–1522, <http://dx.doi.org/10.1890/09-1310.1>
- Villnäs A, Perus J, Bonsdorff E (2011) Structural and functional shifts in zoobenthos induced by organic enrichment - implications for community recovery potential. *Journal of Sea Research* 65: 8–18, <http://dx.doi.org/10.1016/j.seares.2010.06.004>
- Violle C, Navas M-L, Vile D, Kazakou E, Fortunel C, Hummel I, Garnier E (2007) Let the concept of trait be functional. *Oikos* 116: 882–892, <http://dx.doi.org/10.1111/j.0030-1299.2007.15559.x>
- Vitousek PM (1990) Biological invasions and ecosystem processes: towards an integration of population biology and ecosystem studies. *Oikos* 57: 7–13, <http://dx.doi.org/10.2307/3565731>
- Webb TJ, Tyler EHM, Somerfield PJ (2009) Life history mediates large scale population ecology in marine benthic taxa. *Marine Ecology Progress Series* 396: 293–306, <http://dx.doi.org/10.3354/meps08253>
- Weissburg MJ, Zimmer-Faust RK (1993) Life and death in moving fluids: hydrodynamic effects on chemosensory-mediated predation. *Ecology* 74: 1428–1443, <http://dx.doi.org/10.2307/1940072>
- Werner EE, Gilliam JF (1984) The ontogenetic niche and species interactions in size-structured populations. *Annual Review of Ecology and Systematics* 15: 393–425, <http://dx.doi.org/10.1146/annurev.es.15.110184.002141>
- Williams AB (1984) *Shrimps, lobsters and crabs of the Atlantic coast of the eastern United States, Maine to Florida*. Smithsonian Institution Press, Washington DC, 550 pp
- Zaitsev Y, Öztürk B (eds) (2001) *Exotic species in the Aegean, Marmara, Black, Azov and Caspian Seas*. Published by Turkish Marine Research Foundation, Istanbul, Turkey, 267 pp



Laboratory analysis of the habitat occupancy of the crab *Rhithropanopeus harrisi* (Gould) in an invaded ecosystem: The north-eastern Baltic Sea



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ABSTRACT

The Harris mud crab (*Rhithropanopeus harrisi*) arrived to the Baltic Sea in 1936. It was not until the late 2000s when the species considerably expanded its distribution area towards the northern Baltic Sea and formed a viable and expanding population. This introduction represents an appearance of a completely new function, as such larger epibenthic predators were previously missing from north-eastern Baltic Sea. In order to assess potential impacts of the crab to the invaded ecosystem, knowledge of the crab habitat preferences is required. This study experimentally evaluated the habitat occupancy of the Harris mud crab. The crab stayed more in vegetated boulders compared to unvegetated boulders or sandy habitats. There was an interactive effect between the presence of prey and crab population density with prey availability increasing the crab's affinity towards less favored habitats when population densities were low. Increased aggression between crab individuals increased their affinity towards otherwise less occupied habitats. Less favored habitats were typically inhabited by smaller individuals and presence of prey increased occupancy of some habitats for larger crabs. The experiment demonstrated that the crab may inhabit a large variety of habitats with stronger affinity towards boulder fields covered with the brown macroalga *Fucus vesiculosus*. This implies stronger impact of crab in such habitats in the invaded ecosystem.

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1. Introduction

Over the last centuries international trading via large vessels has increased rapidly. Brackish water species, with their ability to survive in ballast water or attached to ship hulls as fouling, are being spread by increasing numbers to previously unreachable suitable habitats on the other side of geographical barriers (Wolff, 1999; Briski et al., 2012). The number of non-indigenous species recorded around harbors is rising (Wolff, 1999; Paavola et al., 2005) and many are becoming invasive for the ecosystems (GISD, 2005).

Motile predators play an important role in marine ecosystems with the top-down control of the abundance and distribution of

benthic species for several trophic levels (Silliman et al., 2004). Among these motile predators, the Harris mud crab *Rhithropanopeus harrisi* (Gould, 1841) is one of the most notorious invasive species globally. The native habitat range of *R. harrisi* is the northwestern part of Atlantic Ocean from Canada to Mexico (Williams, 1984; Projecto-Garcia et al., 2009). *R. harrisi* has all the abilities to be a successful invader. It is small (maximum carapace width 26 mm) omnivorous, euryhaline and has high fecundity. There have been recordings of ovigerous females with a carapace width of less than 8 mm (Turoboyski, 1973; Roche et al., 2007) and females are capable of multiple spawnings from a single mating (Morgan et al., 1983). Earlier field data and our personal observations have shown that adult crabs can survive in fresh water, but larval development is inhibited under 0.5 salinity (Costlow et al., 1966; Turoboyski, 1973; Boyle et al., 2010). Hence *R. harrisi* has invaded brackish waters, coast regions, estuarine areas and inland water bodies throughout the world, including west coast of North

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America, Panama Canal Zone, Venezuela, Europe, Middle East and Japan (Iseda et al., 2007; Brockerhoff and McLay, 2011). The largest invaded inland water bodies include the Caspian Sea, the Black Sea, the Sea of Azov (Zaitsev and Ozturk, 2001) and very recently the larger part of the Baltic Sea (Kotta and Ojaveer, 2012; Fowler et al., 2013). In invaded areas invasive species are known to have numerous effects on the recipient ecosystems (Goodenough, 2010). While impacts of *R. harrisi* are largely unknown, some speculations include competitive interactions between adult crabs and native benthophagous fishes over amphipods and those between crab larvae and zooplankton over phytoplankton and detritus (Zaitsev and Ozturk, 2001). On the other hand, *R. harrisi* represents an additional food source for native demersal fish (Zaitsev and Ozturk, 2001).

In order to establish a viable population in a recipient ecosystem, species must find a suitable niche either within or outside their native range. Low species richness seems to promote the success of establishment of non-indigenous species (Wolff, 1973, 1999; Paavola et al., 2005). This is especially true for the relatively young Baltic Sea basin where an ever-increasing proportion of non-indigenous species is found in the coastal areas inhabited only by a handful of native species. To date the coastal communities still consist of a few post-glacial immigrants and there are many available ecological niches and functions to be filled (Segerstråle, 1957; Ojaveer et al., 2011). Before the arrival of *Rhithropanopeus harrisi*, no crab species has established a reproducing population in the northern Baltic Sea. Adult Chinese mitten crabs (*Eriocheir sinensis* H. Milne Edwards, 1853) are only occasionally found there, but the species poses no threat to the ecosystem due to their low abundance and the inability to reproduce in low salinity waters (Ojaveer et al., 2007). Currently, *R. harrisi* lacks important predators as well as parasites in the Baltic Sea. In some Baltic Sea areas crab remains have been found in the stomachs of local predatory fish (Filuk and Żmudziński, 1965; Fowler et al., 2013); however, due to its occasional occurrence, predation pressure does not shape the current distribution and abundance of *R. harrisi*.

Since predation seems not to control the populations of the mud crab in the northern Baltic Sea, the availability of suitable habitats could be a key factor in controlling the abundance of the species. In its native range *Rhithropanopeus harrisi* prefers to live on oyster beds and silty-sand habitats (Cohen and Carlton, 1995; Milke and Kennedy, 2001; Newell et al., 2007). The large shells provide a necessary shelter from larger predators and its epifauna and juveniles are used as a food source (Newell et al., 2007). In a new environment, however, with different habitat types and without natural predators, the habitat choice of *R. harrisi* is expected to be controlled by other factors. For example, in a recently invaded west coast of North America *R. harrisi* did not inhabit silty habitats and most specimens were found on wooden debris and under rocks (Petersen, 2006; Roche et al., 2007). In the Baltic Sea range the lack of predation and/or competition plausibly enables *R. harrisi* to colonize areas that have low availability of shelter. The species is even considered to be opportunistic in terms of its habitat selection with the crabs spreading to all possible habitats (Fowler et al., 2013). Nevertheless, as population density increases competition within species also increases and consequently less competitive crabs are forced to migrate into less favorable habitats. In addition, food availability is also expected to be a factor in how the crab perceives habitat quality. Due to its omnivorous feeding strategy a mixed diet should result in best growth rate and condition (Buck et al., 2003). The crab should have increased affinity towards habitats with diverse prey communities. Specifically, even though small motile crustaceans have low energy value and the energy used for foraging and consumption lowers their value even more, the intake of minerals and calcium from such food source is

necessary for the natural development of the crab (Hegele-Drywa and Normant, 2009). Habitats, where foraging effort is the smallest could add extra quality to the habitat. In order to assess the potential impacts of crab density and food availability on the crab occupancy among different habitats, experiments are necessary.

Based on the above the aim of the current study was to experimentally evaluate the habitat occupancy of *Rhithropanopeus harrisi* in the north-eastern Baltic Sea. We also determined how the availability of food and the density of *R. harrisi* populations modulate the spread of crabs. In assessing these effects we also addressed the following secondary questions: (1) whether the presence of prey type (non-migratory vs mobile) changes the occupancy of different habitats by *R. harrisi*, and (2) whether food availability and crab density interactively increases or decreases the crab occupancy within different habitats. We assumed that (1) crabs actively choose habitat, (2) elevated density forces smaller crabs to migrate to less favored habitats, and (3) elevated food availability increases the crab occupancy in less favored habitats, the latter effect being strongest with elevated crab density.

2. Materials and methods

The outdoor mesocosm experiment was conducted on the northern shore of Gulf of Riga, the north-eastern Baltic Sea (58°22.10' N 22°58.69' E) in June 2013. In general, the bottom relief of the area is quite flat, gently sloping towards deeper areas. The prevailing substrate types of the bay are a thin layer of slightly silty-sand mixed with pebbles, gravel or boulders. The prevailing depths are between 1 and 4 m. The Gulf of Riga receives fresh water from a huge drainage area and therefore the study site has reduced salinity of 4.0–6.5 and elevated nutrient levels (Kotta et al., 2008). In the mesocosms field conditions were replicated, the coverage of different habitat types reflected natural coverage (Kotta et al., 2008) and crab densities were calculated from natural densities from field observation in Pärnu Bay. The salinity was measured using the Practical Salinity Scale.

The individuals of *Rhithropanopeus harrisi* (24 specimens) were collected from Pärnu Bay at 3 m depth and FDX-B 'Skinny' PIT tags with individual ID numbers were attached to the crab carapaces with superglue. A total of 17% of the crabs were female, which agrees with other invaded area populations where male individuals dominate (Czerniejewski, 2009). No specimens were ovigenous before or at least 2 months after the experiment, as the experiment was conducted in the beginning of June when breeding period had not started. Average carapace width of the crabs was 13.43 mm (max 17.94 and min 7.21 mm), reflecting the realistic size-frequency distribution of crab population in this ecosystem. Due to the aim of this study, to replicate field conditions, we did not use sex and size as covariates in our analyses as in the field different size and sex crabs compete over the same habitats. In the experiment aquaria with the same crab density levels had similar size spectra. Specifically, average crab size in high density aquaria was 11.51 ± 0.84 mm with a difference between largest and smallest crab being 6.27 ± 0.92 mm. Average crab size in medium density aquaria was 10.99 ± 0.72 mm with a difference between largest and smallest crab being 4.75 ± 0.53 mm. The sizes of the crabs between these crab density levels did not differ statistically significantly (paired t-test $p > 0.05$).

Slightly silty-sand sediment, unvegetated boulders and boulders covered with *Fucus vesiculosus* Linnaeus, 1753 and with *Cladophora glomerata* (Linnaeus) Kützing, 1843 were collected adjacent to the experiment site. The amphipod crustaceans *Gammarus* Fabricius, 1775 (mostly the invasive *Gammarus tigrinus* Sexton, 1939) and snails *Theodoxus fluviatilis* (Linnaeus, 1758) were collected from *F. vesiculosus* fronds by shaking the alga. A pilot experiment has

shown that *Rhithropanopeus harrisii* successfully consumes and forages bivalves, gastropods and amphipods, that includes efficiently caching and consuming gammarids. During the experiment visual observations on the movements of prey animals were documented for all aquaria during every measurement. In addition movements of prey animals were documented in additional spare aquaria without crabs. Both gammarids and *T. fluviatilis* snails were selected for the experiment as they are the most common animals in all the habitats.

The experiment was conducted in 12 aquaria (44 l, bottom surface area 0.15 m²), filled by 3/5 with adjacent seawater (21 °C, salinity 6) and approximately 5 cm of slightly silty-sand (hereafter sand). All aquaria were gently aerated throughout the experiment. Boulders with and without macroalgae were distributed uniformly throughout all the aquaria, with the distance between boulders did not exceed 0.15 m. A total of four habitats were generated: boulder with *Fucus vesiculosus*, boulder with *Cladophora glomerata*, unvegetated boulder and sand. The coverage of sand (45%) and boulder with *F. vesiculosus* (25%) exceeded the coverage of two other habitats (each 15%), as in field conditions. Three levels of crab densities were used: 1 = low, 2 = medium and 4 = high. Three types of food conditions were used: no food, presence of non-migratory snails and presence of mobile gammarids (Table 1). In the experimental conditions, when invertebrate prey was added, food was not limiting for crabs and their densities did not fall below natural field densities during the experiment. The crabs were placed randomly in the aquaria and let to acclimate for 24 h. During experiment crabs were located via transmitter tag with Portable FDX/HDX Reader every 2 h for 72 h. In the first of two runs no food and non-migratory food treatments were conducted. In the second run the above set up procedure was repeated with mobile food in 6 aquaria. The crabs used in the second run were from the first run's no food treatment (12 individuals) and randomized prior to emplacement in aquaria. Prior to statistical analysis the rates of occupancy for habitats were adjusted to the amount of crabs per aquarium. In the analyses two aquaria was used for each treatment level and repeated measures were used as replicates, as the crabs moved around sufficiently between two consecutive measurements. In a pilot study we observed that crabs did not exhibit diel cycles in habitat occupancy and the used mesocosms did not differ systematically.

Nevertheless, as temporal dependence may be still present in the dataset, we used a machine learning technique, the Boosted Regression Trees modelling (BRT) to quantify the relative contribution of different treatments on the habitat occupancy of *Rhithropanopeus harrisii* and the model was used to seek the most important separate effects and interactions driving the observed differences. BRT is not sensitive to autocorrelated data, it has no need for prior data transformation or elimination of outliers and can fit complex nonlinear relationships. The BRT also avoids overfitting the data, thereby providing very robust estimates. What is most important in the ecological perspective is that it automatically handles interaction effects between predictors. Due to its strong

predictive performance, BRT is increasingly used in ecology (Elith et al., 2008; Kotta et al., 2013).

BRT models are capable of handling different types of predictor variables and their predictive performance is superior to most traditional modelling methods. The BRT iteratively develop a large ensemble of small regression trees constructed from random subsets of the data. Each successive tree predicts the residuals from the previous tree to gradually boost the predictive performance of the overall model. Although BRT models are complex, they can be summarized in ways that give powerful ecological insight (Elith et al., 2008).

In fitting a BRT the learning rate and the tree complexity must be specified. The learning rate determines the contribution of each successive tree to the final model, as it proceeds through the iterations. The tree complexity fixes whether only main effects (tree complexity = 1) or interactions are also included (tree complexity > 1). Ultimately, the learning rate and tree complexity combined determine the total number of trees in the final model. For both models the model learning rate was kept at 0.01 and interaction depth at 5. Model performance was evaluated using the cross validation statistics calculated during model fitting (Hastie et al., 2009). A random 20% of the data was assigned for testing model accuracy. The BRT modelling was done in the statistical software R using the gbm package (RDC Team, 2013). Altogether two models were run. In the first model independent variables were habitat type, food availability, food type and density of the crabs which were used to separately and interactively predict crab occupancy within a habitat. In the second model the same independent variables were used to predict crab carapace width in a habitat. The latter model describes how the observed factors (habitat type, food and crab density) influence the size-specific distribution among habitats i.e. are the sizes of crabs different among habitat types.

In addition, in order to quantify statistical significances among factor treatments and better visualize the results, the analysis of variance (three-way ANOVA) was performed to investigate the effect of habitat type, food type and crab density on the habitat occupancy and carapace width of the crabs in a habitat. Post-hoc Bonferroni tests were used to analyze which treatment levels differed statistically from each other.

3. Results

The BRT models described 99.8% of the overall variation of the habitat occupancy of *Rhithropanopeus harrisii*. An interaction between habitat, food availability and crab density was the main component of variation with factor level differences explaining more than 95.4% of variability in the habitat occupancy. All other interactions and separate effects of factors had only marginal effects on the habitat occupancy of *R. harrisii*. Similarly, the interaction between habitat, food availability and crab density, was the main component of variation and described 97.4% of variability in the crab carapace width in a habitat, with the BRT model describing close to 100% of the overall variation in the crab carapace width in habitats. In general, the factorial ANOVA analyses supported the results from BRT models with habitat factor ($F_{3, 1788} = 139.96$; $p < 0.001$) and all factor interactions except for density and food ($P_{\text{food} \times \text{density} \times \text{habitat type}} < 0.001$, $F_{12, 1788} = 2.97$; $P_{\text{food} \times \text{habitat type}} < 0.001$, $F_{6, 1788} = 8.92$; $P_{\text{density} \times \text{habitat type}} < 0.001$, $F_{3, 1788} = 12.2$) being statistically significant for the crab habitat occupancy rates (Fig. 1). The differences in the crabs carapace width in different habitats was statistically significantly related to the density of the crabs ($p < 0.001$, $F_{2, 772} = 23.67$) and habitat type ($p < 0.001$, $F_{3, 772} = 7.43$) and all factors interactions except for density and food ($P_{\text{food} \times \text{density} \times \text{habitat type}} < 0.002$, $F_{12, 772} = 2.68$; $P_{\text{food} \times \text{habitat type}}$

Table 1
Experimental treatments – the studied factors and factor levels.

Treatment no	Crab density	Food
1	1	No food
2	2	No food
3	4	No food
4	1	Non-migratory
5	2	Non-migratory
6	4	Non-migratory
7	1	Mobile
8	2	Mobile
9	4	Mobile

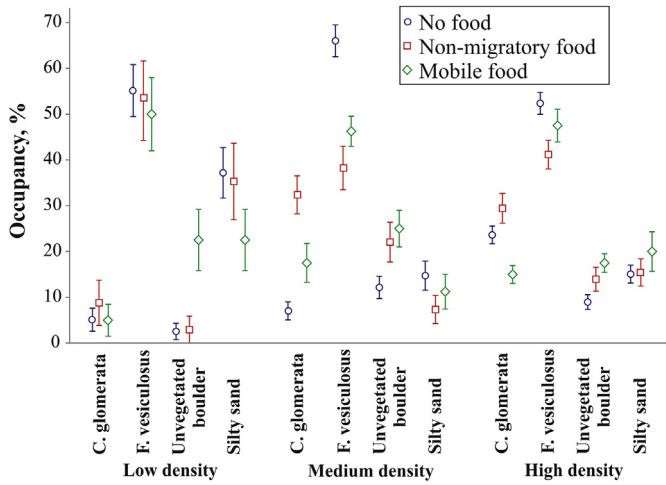


Fig. 1. Average (\pm SE) habitat occupancy of *R. harrisi* within the studied habitats at different crab densities and food types.

type = 0.04, $F_{6, 772} = 2.12$; $p_{\text{density} \times \text{habitat type}} < 0.001$, $F_{3, 772} = 4.35$) (Fig. 2). Thus, below the results of BRT models are presented only if significantly deviating from the ANOVA analyses.

Rhithropanopeus harrisi primarily inhabited boulders as compared to soft bottom habitat. The crabs mostly stayed under boulders covered with *Fucus vesiculosus* (with an average occupancy of 52%) whereas the occupancy within other habitats i.e. sand (20%), boulders covered with *Cladophora glomerata* (15%) or unvegetated boulders (13%) was lower. We observed that within these habitats the mud crab mostly stayed under sediment.

Habitat choice was significantly modulated by separate and interactive effects of crab density and availability of food (Fig. 1). The availability of food only influenced the habitat occupancy at low and medium densities. Specifically, at low crab density the presence of mobile food increased the crab occupancy within unvegetated boulders (important in the BRT models but not significant in the factorial ANOVA) and at medium crab density the presence of non-migratory food decreased the crab occupancy within boulders covered with *Fucus vesiculosus* (Post-hoc

Bonferroni: $p < 0.001$) and increased within boulders with *Cladophora glomerata* (Post-hoc Bonferroni: $p < 0.01$). In the BRT model, however, both types of food decreased the crab occupancy within boulders covered with *F. vesiculosus*. At highest crab densities the availability and/or type of food had no significant impact on the habitat occupancy of *Rhithropanopeus harrisi*. In general, at higher population densities crabs spread more uniformly among different types of habitat compared to low density conditions (Fig. 1).

Different habitat types were generally occupied by different sized crab individuals (Fig. 2). Only in single crab treatments the sizes of the crabs did not significantly vary among habitats (Post hoc Bonferroni: between all habitat types in the absence of food, presence of non-migratory food or presence of mobile food $p > 0.05$). In the presence of competition larger crabs inhabited the most preferred habitat i.e. boulders covered with *Fucus vesiculosus* whereas smaller crabs were found in other habitats in both medium and high density treatments. In both densities habitat factor statistically significantly influenced the sizes of the crabs (Post hoc Bonferroni for medium density: in the absence of food between *F. vesiculosus* and unvegetated boulder habitats ($p < 0.001$) and between silty-sand and unvegetated boulder habitats ($p < 0.001$); for high density: in the absence of food between *F. vesiculosus* and *Cladophora glomerata* habitat ($p < 0.001$) and *C. glomerata* and silty-sand habitat ($p = 0.04$), in the presence of non-migratory food between *F. vesiculosus* and *C. glomerata* habitat ($p = 0.01$), and in the presence of mobile food between *F. vesiculosus* and silty-sand ($p = 0.06$), *F. vesiculosus* and *C. glomerata* ($p < 0.05$) and *F. vesiculosus* and unvegetated boulder habitats ($p < 0.05$). In addition silty-sand habitat was occupied by larger crabs in the presence of intraspecific competition and in the absence of food compared to conditions where food was available (Post-hoc Bonferroni: in medium density between no food and non-migratory food ($p < 0.05$) and between no food and mobile food ($p < 0.05$)). Presence of food did not influence crab sizes in different habitats either with or without intraspecific competition.

The mortality for *Rhithropanopeus harrisi* was under 10% during the experiment. Crabs died only in the treatments associated with the highest crab densities and the absence of animal food. Only larger crabs survived. In these treatments we observed an intense aggression among individuals and usually the smaller dead congeners were immediately consumed.

The visual observations on the movements of prey species showed higher amounts of gammarids and snails near boulders and especially higher amounts of gammarids were observed near *Fucus vesiculosus* and unvegetated boulder habitats. Snails were abundant throughout the aquaria, although they also climbed on aquaria walls, their abundance on the bottom did not fall below their natural densities in field.

4. Discussion

One of the most striking patterns of our study was that throughout the experiment *Rhithropanopeus harrisi* preferred to be under boulders covered with *Fucus vesiculosus* over all other habitats. There was no significant affinity from the crabs towards the algal canopy and they mostly stayed under the rock. It is possible that the flat thallus of *F. vesiculosus* compared to the filamentous structure of *Cladophora glomerata* provides the crab with a stronger shade around the habitat. More shaded habitat in turn offers the crab a better refuge and/or feeding grounds, similarly to e.g. benthic fish (Aneer, 1985). Due to the perenniality and large size, the *F. vesiculosus* habitat provides the invasive crab temporal stability, high species richness and high macroalgal and invertebrate biomasses that no other macrophyte can offer in the Baltic Sea range (Haage, 1976; Kautsky et al., 1992; Wikström and Kautsky, 2007).

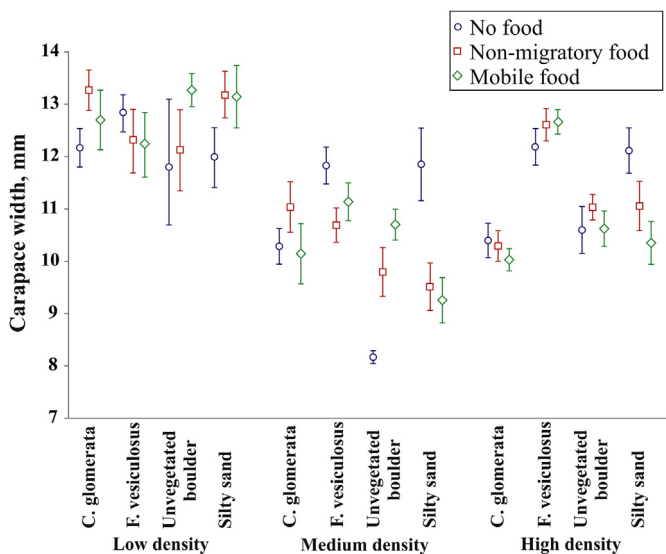


Fig. 2. Average (\pm SE) carapace width of *R. harrisi* within the studied habitats at different crab densities and food types.

Current study showed that when crab densities were low or intermediate the availability and type of food influenced the crab occupancy of different habitats. Mobile prey increased the crab affinity towards unvegetated boulders at lower crab densities. During the experiment visual observations showed that unvegetated boulders and boulders covered with *Fucus vesiculosus* hosted higher densities of gammarid amphipods than silty sediment and boulders covered with *Cladophora glomerata*. In addition to the visual observations, in literature, other amphipods have also shown to avoid open water areas in the presence of predators (Boström and Mattila, 1999) and in field conditions gammarids rapidly colonize all available *F. vesiculosus* thalli (Haage and Jansson 1970). Mobile prey did not induce similar patterns in the crab occupancy of *C. glomerata* habitat. Live *C. glomerata* has low food value and thick cell walls, with makes the algae resistant to herbivory (Birch et al., 1983; Paalme et al., 2002). In addition mesoherbivores have been observed to even become trapped in the filaments of *C. glomerata* and to die there (Orav-Kotta and Kotta, 2004) and this may explain the observed lower abundance of gammarids in *C. glomerata* habitat. Since the availability of food significantly modulated the crab habitat occupancy in both low and intermediate crab density, the habitat selection of the gammarids is possibly behind the habitat occupancy of the crabs in these treatments. Alternatively, low occupancy of the crabs in *C. glomerata* habitat could be explained by possible difficulty in foraging in filamentous algae compared to unvegetated boulders or boulders covered with flat-thalliced *F. vesiculosus*. The extra cost in foraging time is higher with fast moving gammarids (Schoener, 1971; Charnov, 1976), as slow moving snails increased the occupancy of the *C. glomerata* habitat in intermediate crab density. When intraspecific competition increases, the extra effort in foraging in *C. glomerata* habitat outweighs possible aggression effects in other, otherwise favored, habitats.

While intraspecific competition and aggression is not investigated for *Rhithropanopeus harrisii*, competition between *R. harrisii* and slightly larger native crab *Hemigrapsus oregonensis* (Dana, 1851) has forced *R. harrisii* to inhabit other habitats in other invaded areas (Jordan, 1989). While Jordan (1989) found no intraspecific predation of *R. harrisii* adults and only juveniles were consumed by the adult individuals, current study observed aggression among adults that resulted in the consumption of a crab individual. That shows that intraspecific competition and aggression could be an important factor in *R. harrisii* ecology.

Our experiment also indicated that at highest crab densities possible aggression between individuals had a more important effect on the habitat occupancy of crabs than the availability of food. When crab densities increased boulders with *Fucus vesiculosus* were still the most occupied habitats but occupancy of other boulder habitats increased. The experiment demonstrated that at the highest crab density differences in the occupancy rates of all studied habitats were the smallest compared to low crab density conditions, where one habitat is highly occupied and other only marginally. Thus, an increased intraspecific competition among the crabs resulted in broader habitat occupancy. In addition, in the presence of high or medium competition the otherwise less favored habitats were occupied by smaller crab individuals and the most favored habitat, boulders with *F. vesiculosus* was always inhabited by the largest crabs. The same pattern did not occur in conditions without competition. Avoidance of being eaten by the emigration of smaller individuals at high density conditions is also demonstrated by experiments with the Dungeness crab (*Metacarcinus magister* (Dana, 1852)) (Iribarne et al., 1994), the European green crab (*Carcinus maenas* (L.)) (Ropes, 1968) and juvenile blue crabs (*Callinectes sapidus* Rathbun, 1896) (Dittel et al., 1995). Similar behavior has shown to increase the secondary invasion potential for another

benthic invasive species, the round goby (*Neogobius melanostomus* (Pallas, 1814)), whose adults force juveniles to inhabit less favored open sands (Ray and Corkum, 2001). Although larger crab individuals also used open sand habitat in the absence of food and presence of medium competition, which could be attributed to larger individuals searching for food while smaller individuals hide under boulders from possible intraspecific aggression.

The mortality for *Rhithropanopeus harrisii* was low during the experiment. All mortalities were likely a result of intraspecific aggression among crabs occurring only at the highest population densities and the absence of food. Thus, the results suggest that the availability of favorable habitat e.g. boulders covered with *Fucus vesiculosus* may be also one of the factors regulating crab density in field, especially at low food concentrations in early spring when temperatures rise above 10° but densities of prey populations sizes are still at the lowest level.

In conclusion in the north-eastern Baltic Sea *Rhithropanopeus harrisii* is presumed to have low occupancy within unvegetated sand habitat, instead it probably inhabits boulders covered with *Fucus vesiculosus*. Increasing population density could result in a large range expansion and occupancy of less favored habitats, especially when food availability changes. While the habitat occupancy of the mud crab is region specific and therefore has to be determined in each ecosystem, factors examined in this study, can influence the occupancy of different habitats by the crab similarly in all regions. Moreover, whether seasonal changes in prey and/or crab population influence crabs habitat occupancy is yet to be determined. The consequences of the invasion of *R. harrisii* in the north-eastern Baltic Sea can be large at ecosystem levels as *F. vesiculosus* is virtually the only year-round diversity-providing habitat in the northern Baltic Sea. The magnitude and consequence of such impacts need to be determined for both hard bottom and mixed sedimentary habitats.

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References

- Aneer, G., 1985. Some speculations about the Baltic herring (*Clupea harengus membras*) in connection with the eutrophication of the Baltic Sea. *Can. J. Fish. Aquat. Sci.* 42, 83–90.
- Birch, P.B., Gabrielson, J.O., Hamel, K.S., 1983. Decomposition of *Cladophora*. I. Field studies in the Peel-Harvey estuarine system, Western Australia. In: Dring, M. (Ed.), *Botanica Marina*. Walter de Gruyter & Co, Berlin, New York, pp. 165–172.
- Boström, C., Mattila, J., 1999. The relative importance of food and shelter for seagrass-associated invertebrates: a latitudinal comparison of habitat choice by isopod grazers. *Oecologia* 120, 162–170.
- Boyle, J.T., Keith, D., Pfau, R., 2010. Occurrence, reproduction, and population genetics of the estuarine mud crab, *Rhithropanopeus harrisii* (Gould, 1841) (Decapoda, Panopidae) in Texas freshwater reservoirs. *Crustaceana* 83, 493–505.

- Briski, E., Ghabooli, S., Bailey, S.A., MacIsaac, H.J., 2012. Invasion risk posed by macroinvertebrates transported in ships' ballast tanks. *Biol. Invasions* 14, 1843–1850.
- Brockhoff, A., McLay, C., 2011. Human-mediated spread of alien crabs. In: Galil, B.S., Clark, P.F., Carlton, J.T. (Eds.), *In the Wrong Place – Alien Marine Crustaceans: Distribution, Biology and Impacts*. Springer, Netherlands, pp. 27–106.
- Buck, T.L., Breed, G.A., Pennings, S.C., Chase, M.E., Zimmer, M., Carefoot, T.H., 2003. Diet choice in an omnivorous salt-marsh crab: different food types, body size, and habitat complexity. *J. Exp. Mar. Biol. Ecol.* 292, 103–116.
- Charnov, E.L., 1976. Optimal foraging, the marginal value theorem. *Theor. Popul. Biol.* 9, 129–136.
- Cohen, A.N., Carlton, J.T., 1995. Nonindigenous Aquatic Species in a United States Estuary.
- Costlow, J.D., Bookhout, C.G., Monroe, R.J., 1966. Studies on the larval development of the crab, *Rhithropanopeus harrisi* (Gould, 1841). The effect of salinity and temperature on larval development. *Physiol. Zool.* 39, 81–100.
- Czerniejewski, P., 2009. Some aspects of population biology of the mud crab, *Rhithropanopeus harrisi* (Gould, 1841) in the Odra estuary, Poland. *Oceanol. Hydrobiol. Stud.* 38, 49–62.
- Dittel, A.I., Hines, A.H., Ruiz, G.M., Ruffin, K.K., 1995. Effects of shallow water refuge on behavior and density-dependent mortality of juvenile blue crabs in Chesapeake Bay. *Bull. Mar. Sci.* 57, 902–916.
- Eliith, J., Leathwick, J.R., Hastie, T., 2008. A working guide to boosted regression trees. *J. Animal Ecol.* 77, 802–813.
- Filuk, J., Żmudziński, L., 1965. Feeding of the ichthyofauna of the Vistula Lagoon. *Pr. MIR* 14A, 121–147 (in Polish).
- Fowler, A.E., Frosström, T., von Numers, M., Vesakoski, O., 2013. The North American mud crab *Rhithropanopeus harrisi* (Gould, 1841) in newly colonized Northern Baltic Sea: distribution and ecology. *Aquat. Invasions* 8, 89–96.
- GISD, (2005) *Global Invasive Species Database (GISD)*. WWW Page, <http://www.issg.org/database/> (accessed 01.09.05.).
- Goodenough, A.E., 2010. Are the ecological impacts of alien species misrepresented? A review of the “native good, alien bad” philosophy. *Community Ecol.* 11, 13–21.
- Haage, P., Jansson, B.O., 1970. Quantitative investigations of the Baltic Fucus belt macrofauna. *Ophelia* 8, 187–195.
- Haage, P., 1976. Quantitative Investigations of the Baltic Fucus Belt Macrofauna, 3: Seasonal Variation in Biomass, Reproduction and Population Dynamics of the Dominant Taxa. Contributions from the Askoe Laboratory.
- Hastie, T., Tibshirani, R., Friedman, J., 2009. *The Elements of Statistical Learning: Data Mining, Inference and Prediction*. Springer-Verlag, New York, p. 744.
- Hegele-Drywa, J., Normant, M., 2009. Feeding ecology of the American crab *Rhithropanopeus harrisi* (Crustacea, Decapoda) in the coastal waters of the Baltic Sea. *Oceanologia* 51, 361–375.
- Iribarne, O., Fernandez, M., Armstrong, D., 1994. Does space competition regulate density of juvenile dungeness crab *Cancer magister* dana in sheltered habitats? *J. Exp. Mar. Biol. Ecol.* 183, 259–271.
- Iseida, M., Otani, M., Kimura, T., 2007. First record of an introduced crab *Rhithropanopeus harrisi* (Crustacea: Brachyura: Panopeidae) in Japan. *Jpn. J. Benthol.* 62, 39–44.
- Jordan, J.R., 1989. Interspecific Interactions between the Introduced Atlantic Crab *Rhithropanopeus harrisi* and the Native Estuarine Crab *Hemigrapsus oregonensis* in Coos Bay, Oregon (Master of Science thesis). University of Oregon, USA.
- Kautsky, H., Kautsky, L., Kautsky, N., Kautsky, U., Lindblad, C., 1992. Studies on the *Fucus vesiculosus* community in the Baltic Sea. *Acta Phytogeogr. Suec.* 78, 33–48.
- Kotta, J., Ojaveer, H., 2012. Rapid establishment of the alien crab *Rhithropanopeus harrisi* (Gould) in the Gulf of Riga. *Est. J. Ecol.* 61, 293–298.
- Kotta, J., Lauringson, V., Martin, G., Simm, M., Kotta, I., Herkül, K., Ojaveer, H., 2008. Gulf of Riga and Pärnu Bay. In: Schiewer, U. (Ed.), *Ecology of Baltic Coastal Waters, Ecological Studies*, vol. 197. Springer-Verlag, Berlin Heidelberg, pp. 217–243.
- Kotta, J., Kutser, T., Teeveer, K., Vahtmäe, E., Pärnoja, M., 2013. Predicting species cover of marine macrophyte and invertebrate species combining hyperspectral remote sensing, machine learning and regression techniques. *PLoS One* 8, e63946.
- Milke, L.M., Kennedy, V.S., 2001. Mud crabs (Xanthidae) in Chesapeake Bay: claw characteristics and predation on epifaunal bivalves. *Invertebr. Biol.* 120, 67–77.
- Morgan, S.G., Goy, J.W., Costlow, J.D.J., 1983. Multiple ovipositions from single matings in the mud crab *Rhithropanopeus harrisi*. *J. Crustac. Biol.* 3, 542–547.
- Newell, R.I.E., Kennedy, V.S., Shaw, K.S., 2007. Comparative vulnerability to predators, and induced defense responses, of eastern oysters *Crassostrea virginica* and non-native *Crassostrea ariakensis* oysters in Chesapeake Bay. *Mar. Biol.* 152, 449–460.
- Ojaveer, H., Gollasch, S., Jaanus, A., Kotta, J., Laine, A., Minde, A., Normant, M., Panov, V., 2007. Chinese mitten crab *Eriocheir sinensis* in the Baltic Sea—a supply-side invader? *Biol. Invasions* 9, 409–418.
- Ojaveer, H., Kotta, J., Pöllumäe, A., Pöllupüü, M., Jaanus, A., Vetemaa, M., 2011. Alien species in a brackish water temperate ecosystem: annual-scale dynamics in response to environmental variability. *Environ. Res.* 111, 933–942.
- Orav-Kotta, H., Kotta, J., 2004. Food and habitat choice of the isopod *Idotea baltica* in the northeastern Baltic Sea. *Hydrobiologia* 514, 79–85.
- Paalme, T., Kukk, H., Kotta, J., Orav, H., 2002. “In vitro” and “in situ” decomposition of nuisance macroalgae *Cladophora glomerata* and *Pilayella littoralis*. In: Orive, E., Elliott, M., deJonghe, V.N. (Eds.), *Nutrients and Eutrophication in Estuaries and Coastal Waters*. Springer, pp. 469–476.
- Paavola, M., Olenin, S., Leppäkoski, E., 2005. Are invasive species most successful in habitats of low native species richness across European brackish water seas? *Estuar. Coast. Shelf Sci.* 64, 738–750.
- Petersen, C., 2006. Range expansion in the northeast Pacific by an estuary mud crab – a molecular study. *Biol. Invasions* 8, 565–576.
- Projecto-García, J., Cabral, H., Schubart, C.D., 2009. High regional differentiation in a North American crab species throughout its native range and invaded European waters: a phylogeographic analysis. *Biol. Invasions* 12, 253–263.
- Ray, W.J., Corkum, L.D., 2001. Habitat and site affinity of the round goby. *J. Gt. Lake Res.* 27, 329–334.
- RDC Team, 2013. *R: a Language and Environment for Statistical Computing*. WWW Page. <http://www.R-project.org/> (accessed 20.07.13.).
- Roche, D., Torchin, M., Leung, B., Binning, S., 2007. Localized invasion of the North American Harris mud crab, *Rhithropanopeus harrisi*, in the Panama Canal: implications for eradication and spread. *Biol. Invasions* 11, 983–993.
- Ropes, J.W., 1968. The feeding habits of the green crab, *Carcinus maenas* (L.). *Fish. Bull.* 67, 183–203.
- Schoener, T.W., 1971. Theory of feeding strategies. *Annu. Rev. Ecol. Syst.* 2, 369–404.
- Seegerstråle, S.G., 1957. Baltic Sea. In: *Geological Society of America Memoirs*, vol. 67, pp. 751–800.
- Silliman, B.R., Layman, C.A., Geyer, K., Zieman, J., 2004. Predation by the black-clawed mud crab, *Panopeus herbstii*, in mid-Atlantic salt marshes: further evidence for top-down control of marsh grass production. *Estuaries* 27, 188–196.
- Turobovski, K., 1973. Biology and ecology of the crab *Rhithropanopeus harrisi* ssp. *tridentatus*. *Mar. Biol.* 23, 303–313.
- Wikström, S.A., Kautsky, L., 2007. Structure and diversity of invertebrate communities in the presence and absence of canopy-forming *Fucus vesiculosus* in the Baltic Sea. *Estuar. Coast. Shelf Sci.* 72, 168–176.
- Williams, A.B., 1984. *Shrimps, Lobsters, and Crabs of the Atlantic Coast of the Eastern United States, Maine to Florida*. Smithsonian Institution Press, Washington D.C., p. 550.
- Wolff, W.J., 1973. The estuary as a habitat: an analysis of data on the soft-bottom macrofauna of the estuarine area of the rivers Rhine, Meuse and Scheldt. *Zool. Verhhandelingen* 126, 3–242.
- Wolff, W.J., 1999. Exotic invaders of the meso-oligohaline zone of estuaries in the Netherlands: why are there so many? *Helgol. Meeresunters.* 52, 393–400.
- Zaitsev, Y., Ozturk, B. (Eds.), 2001. *Exotic Species in the Aegean, Marmara, Black, Azov and Caspian Seas*. Turkish Marine Research Foundation, Istanbul Turkey, p. 267.

Invasive Harris mud crab (*Rhithropanopeus harrisi*, Gould 1841) prefers isopod prey in the Northern Baltic Sea

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A taste for aliens: Incorporation of a novel prey item into native fishes diet

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The Grass Prawn (*Palaemon elegans*)



Trophic interactions between native and alien palaemonid prawns and an alien gammarid in a brackish water ecosystem

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Abstract. Macroalgae are an important habitat for small mobile invertebrates such as gammarid amphipods and palaemonid prawns. Gammarid amphipods are important grazers of micro- and macroalgae whereas palaemonid prawns are feeding on macroalgae and small aquatic invertebrates including gammarids. Recently the invasive palaemonid prawn *Palaemon elegans* established in the Baltic Sea. As *P. elegans* occurs within the same habitats as the native *Palaemon adspersus*, it is expected that this invasion modifies the existing trophic interactions. To address this question, we experimentally investigated the feeding of the native *P. adspersus* and the invasive *P. elegans* on the benthic macroalga *Cladophora glomerata* and on the invasive gammarid amphipod *Gammarus tigrinus*. In the course of the experiment neither *G. tigrinus* nor *Palaemon* spp. had effects on filamentous macroalgae. The presence of prawns drastically increased the mortality of amphipods with no difference in the feeding efficiency between the two prawn species. To conclude, the alien prawn does not add an extra function to the trophic system of the coastal ecosystem of the Baltic Sea. Nevertheless, due to its progressively increasing densities and wide habitat range, *P. elegans* is expected to exert stronger predation pressure on gammarid amphipods as compared to *P. adspersus* alone.

Key words: Baltic Sea, invasive species, *Palaemon elegans*, *Palaemon adspersus*, *Gammarus tigrinus*, trophic interactions, predation.

INTRODUCTION

In aquatic systems with low species diversity, predation can be a strong structuring force (Flecker and Townsend, 1994; Worm and Myers, 2003), and the addition of an efficient predator may result in many direct and indirect effects leading to a reorganization of the entire food web (Carpenter et al., 1985). Nowadays the rates of bioinvasions are progressively increasing in the coastal seas around the world with decapod crustaceans being amongst the most notorious pests (Weis, 2011). When established, such predators may generate changes in trophic interactions of invaded systems equivalent to for instance coastal eutrophication or climate change (e.g. Grosholz et al., 2000). Although native species are better adapted to their environment, invasive species

may outcompete native species as they are often better foragers (Weis, 2011). When present at high numbers, predators may drastically reduce populations of herbivores and thereby indirectly facilitate blooms of ephemeral algae in coastal ecosystems (Eriksson et al., 2009; Sieben et al., 2011). Moreover, in the absence of invertebrate prey, introduced decapods may also feed on some macroalgal species and thereby cause shifts in the macroalgal community structure.

The invasive rockpool prawn *Palaemon elegans* established almost in the entire Baltic Sea recently (Janas and Maňkucka, 2010; Katajisto et al., 2013). Its Baltic population is genetically closer to the Mediterranean, Black Sea, and Caspian Sea populations than to the Atlantic population (Reuschel et al., 2010). This evidence clearly suggests that *P. elegans* is a non-native species in the Baltic Sea, which has been introduced by human activity. The ecological consequences

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of the large-scale and rapid invasion of *P. elegans* are barely known.

As the Baltic Sea is characterized by low numbers of benthic invertebrate predators and the invasive palaemonid has attained high densities in numerous habitats, the species is expected to have an effect on the food webs of the coastal sea. Earlier studies have shown that palaemonid prawns can feed on both benthic macroalgae and invertebrates (Hartnoll and Salama, 1992; Janas and Barańska, 2008; Moksnes et al., 2008; Lesutienė et al., 2014) with amphipods serving as their main food (Möller et al., 1985; Persson et al., 2008). As *P. elegans* occurs within the same habitats as the native *Palaemon adspersus*, the invasive palaemonid may outcompete the native palaemonid species. It is also expected that this invasion may modify the existing trophic interactions either due to the addition of a new function (i.e. preying on different organisms compared to the native prawn species) or intensification of the existing interactions (e.g. increasing predation pressure on amphipods). Additionally, *P. elegans* is also a valuable food for fish (Janas and Bruska, 2010; Gruszka and Więcaszek, 2011). As the effects of the prawn are expected to be context specific (Smaldon, 1979; Berglund, 1980), it is not possible to extrapolate findings of previous studies over the entire Baltic Sea.

It is difficult to assess the strength of interspecific interactions in a food web using only field observations because multiple types of interactions act at the same time (Scheffer et al., 2005; Speckman et al., 2005) and several prey and predator species are involved (Chapin et al., 1997; Pinnegar et al., 2000; Frank et al., 2007). Natural communities are complex systems with multiple trophic levels and nonlinear interactions between species. These interactions together with environmental variability can enhance or weaken the separate effects of a single species of interest (Schmitz, 2007). In the context of palaemonid invasion this can obscure the true effect of *P. elegans*.

Experimental studies can effectively reveal cause–effect relationships in a multitrophic system and assess intensities of various controls involved (Hunt and McKinnell, 2006). Although experiments where vegetation, herbivores, and predators are simultaneously manipulated can be regarded as an important tool to assess the relative strength of trophic interactions and cascade effects, such studies are still rare (e.g. Korpinen et al., 2007; Moksnes et al., 2008; Persson et al., 2008; Wernberg et al., 2013). Nevertheless, all these studies have specifically demonstrated critical roles of predation on invertebrate prey and macroalgae that would not have been revealed if only two trophic levels had been included into the experimental design.

In the current study we experimentally manipulated the presence of the filamentous macroalga *Cladophora glomerata*, the invasive amphipod *Gammarus tigrinus*,

the native prawn *P. adspersus*, and the invasive prawn *P. elegans* in order to test (1) whether *P. elegans* had different grazing and/or predation rates than *P. adspersus* and (2) whether the grazing and/or predation rates varied among mixed and single species assemblages. Based on earlier evidence, we expected that gammarid amphipods constitute an important food source for both prawn species. We also expected that due to its higher mobility (Berglund, 1980), the invasive prawn exerted stronger predation pressure on amphipods than the native species and due to its higher aggressiveness and higher efficiency in predator avoidance (Berglund and Bengtsson, 1981), it might induce elevated mortality in the native palaemonid. Finally, we predicted that due to the complementarity effect, assemblages containing both prawn species had higher grazing and/or predation rates compared to assemblages containing a single prawn species.

Methods

An outdoor aquarium experiment was performed adjacent to the Kõiguste Marine Biology Laboratory, the north-eastern Baltic Sea, in August 2012. Experimental organisms were collected from the northern (58°37.34'N, 22°51.78'E) and the southern coast (58°22.25'N, 22°58.77'E) of Saaremaa Island, north-eastern Baltic Sea. The palaemonid prawns *P. adspersus* and *P. elegans* and their prey *G. tigrinus* were collected with hand nets between 0 and 1 m depth. Only adult specimens were used in the experiment. Boulders overgrown with the green filamentous macroalga *Cladophora glomerata* were collected from the same areas. Prior to the beginning of the experiment, all invertebrates were removed from the algae and boulders by vigorously shaking the boulders in filtered sea water followed by a thorough visual inspection. Boulders with filamentous algae in the experiment served as an object of grazing for the palaemonid prawns and gammarid amphipods and as a refuge for gammarids.

The experiment was performed in 5-L aquaria filled with filtered surface water (salinity 5.5) from Kõiguste Bay. The following treatments and treatment levels were used: *Palaemon* community (*P. elegans*, *P. adspersus*, mixed, none), *Palaemon* density (1 or 2 individuals per aquarium, which corresponded to 14 and 28 ind m⁻² or 1.49±0.92 and 2.57±1.06 g m⁻² of dry biomass (mean±standard deviation), respectively), presence of macroalgae (vegetated boulders, unvegetated boulders), presence of gammarid amphipods (present, absent). Altogether 92 aquaria were used to deploy 23 treatments replicated four times (Table 1). The initial density of gammarids within the aquaria was 141 ind m⁻², the cover of macroalgae was set at 50% corresponding to the algal biomass of 3.43±0.85 g dw m⁻². Similar

Table 1. Experimental setup. Four replicates were analysed for each treatment

Treatment	Presence of vegetation	Presence of gammarids	<i>Palaemon</i> community	<i>Palaemon</i> density
1	No	No	<i>P. elegans</i> + <i>P. adspersus</i>	2
2	No	No	<i>P. elegans</i>	1
3	No	No	<i>P. elegans</i>	2
4	No	No	<i>P. adspersus</i>	1
5	No	No	<i>P. adspersus</i>	2
6	No	Yes	<i>P. elegans</i> + <i>P. adspersus</i>	2
7	No	Yes	<i>P. elegans</i>	1
8	No	Yes	<i>P. elegans</i>	2
9	No	Yes	<i>P. adspersus</i>	1
10	No	Yes	<i>P. adspersus</i>	2
11	No	Yes	None	0
12	Yes	No	<i>P. elegans</i> + <i>P. adspersus</i>	2
13	Yes	No	<i>P. elegans</i>	1
14	Yes	No	<i>P. elegans</i>	2
15	Yes	No	<i>P. adspersus</i>	1
16	Yes	No	<i>P. adspersus</i>	2
17	Yes	No	None	0
18	Yes	Yes	<i>P. elegans</i> + <i>P. adspersus</i>	2
19	Yes	Yes	<i>P. elegans</i>	1
20	Yes	Yes	<i>P. elegans</i>	2
21	Yes	Yes	<i>P. adspersus</i>	1
22	Yes	Yes	<i>P. adspersus</i>	2
23	Yes	Yes	None	0

values have been previously observed in the Kõiguste Bay area (Lauringson and Kotta, 2006). The density of *P. adspersus* and *P. elegans* was slightly higher in aquaria than in the field; however, when prawns are swarming in the field, the values match the experimental densities.

The wet weight of algae was determined prior to the experiment to the nearest 0.01 g. Before weighing, the algae and boulders were gently dried on plotting paper until the paper did not become wet any more. At the end of the experiment algae were removed from the boulders, and the boulders and algae were weighed separately. The initial weight of the algae was calculated by subtracting the weight of a boulder. Additional four replicates of boulders with algae were collected to obtain the ratio of wet to dry weight of algae. These algae were dried at 60°C for 48 h. The ratio of wet to dry weight of algae was used to convert the initial algal wet weight to dry weight.

The experiment lasted 48 h. The number of gammarid individuals was visually estimated in every 12 h. The experiment was terminated when at least in one experimental treatment about 50% of gammarids had been consumed. This was necessary to avoid total consumption of gammarids by prawns and to prevent

getting biased estimates of predation rate due to the changing prey densities. During the course of the experiment, the water temperature varied between 14 and 23°C following diel variation in the seawater temperature in Kõiguste Bay.

At the end of the experiment the test animals were counted and determined to the species level. In addition, the body length of prawns was measured from the tip of the rostrum to the tip of the telson. The total body length of palaemonid prawns used in the experiment was as follows (min, mean, max; mm): *P. elegans* 21.7, 38.5, and 49.5; *P. adspersus* 22.3, 38.7, and 51.0. The survival was calculated as the percentage of individuals of *G. tigrinus* and palaemonid prawns that were alive at the end of the experiment. In addition, all remaining algae were collected from the boulders and their dry weight was determined. The algal consumption was quantified as the change in the dry weight of algae between the start and the end of the experiment.

Factorial ANOVA with the density of prawns nested within the *Palaemon* community was used to investigate the separate and interactive effects of treatments on the macroalgal consumption and the survival of amphipods and palaemonid prawns. Post-hoc Bonferroni tests were used to analyse which treatment levels were statistically different from each other. Prior to analyses we checked the validity of the assumptions of ANOVA.

RESULTS

Only one prawn out of 128 specimens died. This indicates that experimental treatments had no clear effects on prawn mortality during the experiment.

During the course of the experiment, we observed that the studied *Palaemon* species did not graze on the filamentous *C. glomerata* (Table 2). Neither did prawns

Table 2. Three-way ANOVA on the effects of gammarids (factor levels: absent, present), *Palaemon* community (factor levels: no prawn, *P. elegans*, *P. adspersus*, mixed community of *P. elegans* and *P. adspersus*), and *Palaemon* density (factor levels: 0, 1, 2 individuals per aquarium) on the macroalgal consumption

Effect	SS	DF	MS	F	p
Gammarids	0.00	1	0.00	0.00	0.97
<i>Palaemon</i> community	0.05	3	0.02	1.20	0.32
Gammarids × <i>Palaemon</i> community	0.05	3	0.02	1.16	0.34
<i>Palaemon</i> density (nested in <i>Palaemon</i> community)	0.02	2	0.01	0.79	0.46
Gammarids × <i>Palaemon</i> density (nested in <i>Palaemon</i> community)	0.03	2	0.01	1.11	0.34
Error	0.48	36	0.01		

indirectly affect the biomass of macroalgae through the removal of the mesoherbivore *G. tigrinus*. Moreover, there were no differences in the algal consumption between single and mixed species prawn treatments and between different palaemonid densities (Table 2).

Prawns significantly reduced the survival of gammarids (Table 3, Table 4, Fig. 1). Over 90% of the gammarid amphipods survived when prawns were absent. In the presence of the predatory palaemonid, the survival of gammarids was reduced to 55%. There was no difference in the feeding activity between the two studied prawn species and the presence of macroalgae did not modify the feeding rates of palaemonid prawns (Table 3). Moreover, the predation of prawns on gammarid amphipods did not vary among single and mixed *Palaemon* species treatments (Table 4) and prawn density had no effect on the survival of *G. tigrinus*.

Table 3. Three-way ANOVA on the effects of vegetation (factor levels: absent, present), *Palaemon* community (factor levels: no prawn, *P. elegans*, *P. adspersus*, mixed community of *P. elegans* and *P. adspersus*), and *Palaemon* density (factor levels: 0, 1, 2 individuals per aquarium) on the survival of gammarids. Statistically significant effect ($p < 0.05$) is indicated in bold

Effect	SS	DF	MS	F	p
Vegetation	485	1	485	0.76	0.39
<i>Palaemon</i> community	9 189	3	3 063	4.77	0.007
Vegetation × <i>Palaemon</i> community	245	3	82	0.13	0.94
<i>Palaemon</i> density (nested in <i>Palaemon</i> community)	625	2	312	0.49	0.62
Vegetation × <i>Palaemon</i> density (nested in <i>Palaemon</i> community)	81	2	41	0.06	0.94
Error	23 772	37	643		

Table 4. Bonferroni post hoc comparisons between the factor levels of *Palaemon* community following three-way ANOVA presented in Table 3. Statistically significant differences ($p < 0.05$) are indicated in bold

	<i>P. elegans</i>	<i>P. adspersus</i>	No prawn
Mixed community	1.00	1.00	0.01
<i>P. elegans</i>		1.00	0.04
<i>P. adspersus</i>			0.02

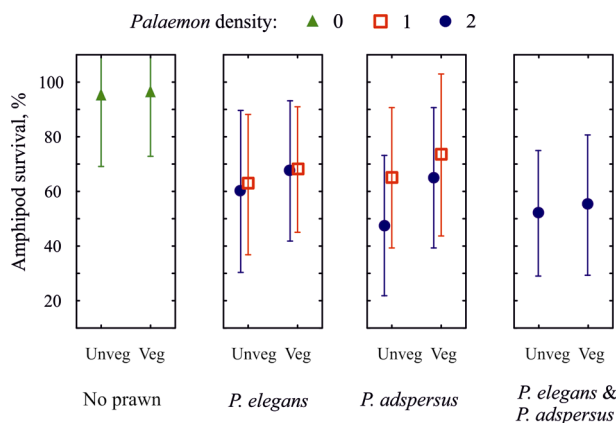


Fig. 1. Effect of vegetation (factor levels: absent, present; denoted as unveg and veg), *Palaemon* community (factor levels: no prawn, *P. elegans*, *P. adspersus*, mixed community of *P. elegans* and *P. adspersus*) and *Palaemon* density (factor levels: 0, 1, 2 individuals per aquarium) on gammarid survival. Vertical bars denote 0.95 confidence intervals.

DISCUSSION

Our experiment demonstrated that *P. elegans* represented a similar function as *P. adspersus*, the only palaemonid previously present in the ecosystem of the north-eastern Baltic Sea. Both palaemonid prawns exerted a strong predatory pressure on gammarid amphipods. Contrastingly, the impacts of *P. elegans* on the native *P. adspersus* and on the macroalga *C. glomerata* were negligible. This suggests that the invasion does not necessarily lead to the reorganization of trophic interactions in the coastal sea unless *P. elegans* colonizes areas where *P. adspersus* is absent or found at low densities. Nevertheless, *P. elegans* tolerates hypoxia and low salinity better (Taylor and Spicer, 1987; Janas et al., 2013) and establishes in areas previously uninhabited by native palaemonid prawns or other invertebrate predators of similar size, for example in the Vistula Lagoon in the southern Baltic Sea (Ezhova et al., 2005) and in the eastern part of the Gulf of Finland in the northern Baltic Sea (I. Kuprijanov, unpublished material). Consequently, in such habitats the invasive palaemonid presents a new function and thereby affects the stability of local food webs (Long et al., 2011).

It has been suggested that in case of a lack of prey, palaemonid prawns can switch to an algal diet (Jephson et al., 2008). Our study suggested otherwise but this may reflect the short time frame of our experiment. In the southern Baltic Sea, the diet of *P. elegans* regularly consists of filamentous algae including *Cladophora* spp. (Janas and Barańska, 2008). However, the volume of animal food always exceeds algal food at the southern Baltic coast (Janas and Barańska, 2008), and the proportion of filamentous algae in the food of *P. adspersus*

and *P. elegans* may differ largely between areas (Jephson et al., 2008).

In the present study, no strong effect of vegetation on gammarid survival emerged. This certainly cannot hint the lack of such effect in the field, where conditions for escapee are substantially better than in a spatially limited aquarium. Normally, the native *P. adspersus* does not inhabit unvegetated areas whereas *P. elegans* can be found in the full range of coastal habitats regardless of macrophyte cover (Katajisto et al., 2013). Thus, when *P. elegans* reaches high densities in unvegetated habitats, it may exert an elevated predatory pressure on gammarid amphipods in such habitats.

In the present experiment, predator density had no effect on gammarid mortality. Gammarids may have reduced their time spent out of hiding places in response to higher prawn densities or alternatively, actively swimming gammarids may have been cropped at efficiencies indifferent of predator densities in the studied range. This may indicate that palaemonid food was a limiting factor at both studied shoaling densities and possibly even at lower densities than observed in the present study. Our study suggests that if a shoal of prawns with the density within the studied range (14–28 ind m⁻²) remained at a place for about 48 h, local gammarid population would be reduced by approximately 50% and the palaemonids might have to change their feeding grounds to meet their nutritional demands.

Previous laboratory experiments have demonstrated that *P. elegans* is able to effectively prey on gammarids (*G. locusta*) less than 8 mm in total length (Persson et al., 2008). In our study, slightly larger amphipods were successfully consumed by both species of palaemonid prawns. In predatory crustaceans, the size of chela defines the range of size of invertebrate prey (Mariappan et al., 2000). Individuals with bigger chelae are expected to attack larger prey (e.g. Elner, 1980), whereas those with smaller chelae catch fast-moving prey and exhibit more generalistic feeding patterns (Seed and Hughes, 1995; Yamada and Boulding, 1998). In addition to having relatively larger chelae, *P. adspersus* has also been shown to grow slightly faster and bigger compared to *P. elegans* (Berglund, 1980); however, the size difference seemed insufficient to cause an effect in our study. As to finding immobile food items *P. elegans* has been shown to be quicker than *P. adspersus* (Berglund, 1980). In our experiment, however, both species caught live amphipods with equal efficiency and differences between single and mixed species treatments were insignificant. Still, in mixed species treatments, food intake by one given prawn species may have been changed by the vicinity of the other species.

Although gammarids were heavily consumed by prawns in our study, it seems possible that *G. tigrinus*

can compensate for high mortality by a very broad reproduction period, high reproduction rate, and short development time. The native gammarids have a later start of breeding, smaller brood size, and longer maturation time (Kotta et al., 2010; Sareyka et al., 2011; Jänes et al., 2015). Therefore, the expanding *P. elegans* population could have stronger effects on native gammarids than on *G. tigrinus*. The invasive gammarid, in turn, may potentially facilitate the invasive predator by offering a more abundant food source than the less productive native prey species. The distribution range and abundance of native gammarids have already decreased due to negative effects of the invasive *G. tigrinus* (Kotta et al., 2010) and the invasion of *P. elegans* may further suppress the populations of native gammarid species.

Two species with very similar ecological niches are expected to either partition their limiting resources or not to occur sympatrically (MacArthur and Levins, 1967). In the North Sea, both prawn species share the range but partition their habitats (Berglund and Bengtsson, 1981). In the Gulf of Gdansk, the southern Baltic Sea, the distribution patterns show a retreat of *P. adspersus* from extensive areas after the invasion of *P. elegans* (Grabowski, 2006). In the north-eastern Baltic Sea, however, the two prawn species coexist in soft and hard bottom habitats and within a wide range of depths (Katajisto et al., 2013; Kuprijanov and Kotta, 2013). Contrary to the southern Baltic Sea, vegetated areas are widespread in the shallow habitats of the north-eastern Baltic Sea and are highly trophic. Therefore, the two prawn species may likely benefit from abundant food resources and accordingly weak interspecific competition in their sympatric summer habitat. It may be that prawns are only limited by predation in their wintering areas (Pihl, 1982; Gruszka and Więcaszek, 2011). Moreover, our experiment was focussed on the gammarid–palaemonid interactions whereas in field conditions the decapod predators are exposed to multiple prey species and are themselves preyed by fish. Thus, further community level, longer-term, and seasonally explicit experiments would provide more detailed knowledge on the roles of palaemonid prawns in the Baltic Sea coastal ecosystems.

To conclude, our study demonstrated how an introduction of a closely related species strengthens an existing function of a palaemonid predator in a food web. Both palaemonid species strongly preferred gammarids over filamentous green algae and preyed on invertebrates at an equal rate. Our results hint that the predatory function of the invasive palaemonid may largely overlap that of the local species, which could lead to niche partitioning or outcompeting processes between these two species. Additionally, the larger habitat range of the invasive species may intensify the top-down control of crustacean herbivores in an

extensive range of coastal areas of the Baltic Sea presently lacking such predator.

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REFERENCES

- Berglund, A. 1980. Niche differentiation between two littoral prawns in Gullmar Fjord, Sweden: *Palaemon adspersus* and *P. squilla*. *Ecography*, **3**, 111–115.
- Berglund, A. and Bengtsson, J. 1981. Biotic and abiotic factors determining the distribution of two prawn species: *Palaemon adspersus* and *P. squilla*. *Oecologia*, **49**, 300–304.
- Carpenter, S. R., Kitchell, J. F., and Hodgson, J. R. 1985. Cascading trophic interactions and lake productivity. *BioScience*, **35**, 634–639.
- Chapin, F. S. III, Walker, B. H., Hobbs, R. J., Hooper, D. U., Lawton, J. H., Sala, O. E., and Tilman, D. 1997. Biotic control over the functioning of ecosystems. *Science*, **277**, 500–504.
- Elnor, R. W. 1980. The influence of temperature, sex and chela size in the foraging strategy of the shore crab, *Carcinus maenas* (L.). *Mar. Behav. Physiol.*, **7**, 15–24.
- Eriksson, B. K., Ljungren, L., Sandström, A., Johansson, G., Mattila, J., Rubach, A., et al. 2009. Declines in predatory fish promote bloom-forming macroalgae. *Ecol. Appl.*, **19**, 1975–1988.
- Ezhova, E., Żmudziński, L., and Maciejewska, K. 2005. Long-term trends in the macrozoobenthos of the Vistula Lagoon, southern Baltic Sea. Species composition and biomass distribution. *Bull. Sea Fisheries Inst.*, **1**, 54–73.
- Flecker, A. S. and Townsend, C. R. 1994. Community-wide consequences of trout introduction in New Zealand streams. *Ecol. Appl.*, **4**, 798–807.
- Frank, K. T., Petrie, B., and Shackell, N. L. 2007. The ups and downs of trophic control in continental shelf ecosystems. *Trends Ecol. Evol.*, **22**, 236–242.
- Grabowski, G. 2006. Rapid colonization of the Polish Baltic coast by an Atlantic palaemonid shrimp *Palaemon elegans* Rathke, 1837. *Aquatic Invasions*, **1**, 116–123.
- Grosholz, E. D., Ruiz, G. M., Dean, C. A., Shirley, K. A., Maron, J. L., and Connors, P. G. 2000. The impacts of a nonindigenous marine predator in a California bay. *Ecology*, **81**, 1206–1224.
- Gruszka, P. and Więcaszek, B. 2011. *Palaemon elegans* Rathke, 1837 in the food of Baltic cod (*Gadus morhua callarias* L., 1758) from the Gulf of Gdańsk. *Mar. Biol. Res.*, **7**, 100–105.
- Hartnoll, R. G. and Salama, A. J. 1992. The effect of protein source on the growth of the prawn *Palaemon elegans* Rathke, 1837 (Decapoda, Caridea). *Crustaceana*, **63**, 81–90.
- Hunt, G. L. Jr. and McKinnell, S. 2006. Interplay between top-down, bottom-up, and wasp-waist control in marine ecosystems. *Prog. Oceanogr.*, **68**, 115–124.
- Janas, U. and Barańska, A. 2008. What is the diet of *Palaemon elegans* Rathke, 1837 (Crustacea, Decapoda), a non-indigenous species in the Gulf of Gdańsk (southern Baltic Sea)? *Oceanologia*, **50**, 222–237.
- Janas, U. and Bruska, O. 2010. Energy values and energy resources of two prawns in Baltic coastal waters: the indigenous *Palaemon adspersus* and the non-indigenous *Palaemon elegans*. *Oceanologia*, **52**, 281–297.
- Janas, U. and Mańkucka, A. 2010. Body size and reproductive traits of *Palaemon elegans* Rathke, 1837 (Crustacea, Decapoda), a recent colonizer of the Baltic Sea. *Oceanol. Hydrobiol. Stud.*, **39**, 3–24.
- Janas, U., Piłka, M., and Lipińska, D. 2013. Temperature and salinity requirements of *Palaemon adspersus* Rathke, 1837 and *Palaemon elegans* Rathke, 1837. Do they explain the occurrence and expansion of prawns in the Baltic Sea? *Mar. Biol. Res.*, **9**, 293–300.
- Jänes, H., Kotta, J., and Herkül, K. 2015. High fecundity and predation pressure of the invasive *Gammarus tigrinus* cause decline of indigenous gammarids. *Estuar. Coast. Shelf S.*, in press.
- Jephson, T., Nyström, P., Moksnes, P.-O., and Baden, S. P. 2008. Trophic interactions in *Zostera marina* beds along the Swedish coast. *Mar. Ecol. Prog. Ser.*, **369**, 63–76.
- Katajisto, T., Kotta, J., Lehtiniemi, M., Malavin, S. A., and Panov, V. E. 2013. *Palaemon elegans* Rathke, 1837 (Caridea: Palaemonoidea: Palaemonidae) established in the Gulf of Finland, the north-eastern Baltic Sea. *BioInvasions Records*, **2**, 125–132.
- Korpinen, S., Jormalainen, V., and Honkanen, T. 2007. Bottom-up and cascading top-down control of macroalgae along a depth gradient. *J. Exp. Mar. Biol. Ecol.*, **343**, 52–63.
- Kotta, J., Orav-Kotta, H., and Herkül, K. 2010. Separate and combined effects of habitat-specific fish predation on the survival of invasive and native gammarids. *J. Sea Res.*, **64**, 369–372.
- Kuprijanov, I. and Kotta, J. 2013. First evidence on the epiphytic macroalga *Pylaiella littoralis* on the prawn *Palaemon adspersus*. *Estonian J. Ecol.*, **62**, 287–291.
- Lauringson, V. and Kotta, J. 2006. Influence of the thin drift algal mats on the distribution of macrozoobenthos in Kõiguste Bay, NE Baltic Sea. *Hydrobiologia*, **554**, 97–105.
- Lesutienė, J., Gasiūnaitė, Z., Strikaitytė, R., and Žilienė, R. 2014. Trophic position and basal energy sources of the invasive prawn *Palaemon elegans* in the exposed littoral of the SE Baltic Sea. *Aquatic Invasions*, **9**, 37–45.

- Long, Z. T., Bruno, J. F., and Duffy, J. 2011. Food chain length and omnivory determine the stability of a marine subtidal food web. *J. Anim. Ecol.*, **80**, 586–594.
- MacArthur, R. and Levins, R. 1967. The limiting similarity, convergence, and divergence of coexisting species. *Am. Nat.*, **101**, 377–385.
- Mariappan, P., Balasundaram, C., and Schmitz, B. 2000. Decapod crustacean chelipeds: an overview. *J. Bioscience*, **25**, 301–313.
- Moksnes, P. O., Gullström, M., Tryman, K., and Baden, S. 2008. Trophic cascades in a temperate seagrass community. *Oikos*, **117**, 763–777.
- Möller, P., Pihl, L., and Rosenberg, R. 1985. Benthic faunal energy flow and biological interaction in some shallow marine soft bottom habitats. *Mar. Ecol. Prog. Ser.*, **27**, 109–121.
- Persson, M., Andersson, S., Baden, S., and Moksnes, P. O. 2008. Trophic role of the omnivorous grass shrimp *Palaemon elegans* in a Swedish eelgrass system. *Mar. Ecol. Prog. Ser.*, **371**, 203–212.
- Pihl, L. 1982. Food intake of young cod and flounder in a shallow bay on the Swedish west coast. *Neth. J. Sea Res.*, **15**, 419–432.
- Pinnegar, J. K., Polunin, N. V. C., Francour, P., Badalamenti, F., Chemello, R., Harmelin-Vivien, M. L., et al. 2000. Trophic cascades in benthic marine ecosystems: lessons for fisheries and protected-area management. *Environ. Conserv.*, **27**, 179–200.
- Reuschel, S., Cuesta, J. A., and Schubart, C. D. 2010. Marine biogeographic boundaries and human introduction along the European coast revealed by phylogeography of the prawn *Palaemon elegans*. *Mol. Phylogenet. Evol.*, **55**, 765–775.
- Sareyka, J., Kraufvelin, P., Lenz, M., Lindström, M., Tollrian, R., and Wahl, M. 2011. Differences in stress tolerance and brood size between a non-indigenous and an indigenous gammarid in the northern Baltic Sea. *Mar. Biol.*, **158**, 2001–2008.
- Scheffer, M., Carpenter, S., and Young, B. D. 2005. Cascading effects of overfishing marine systems. *Trends Ecol. Evol.*, **20**, 579–581.
- Schmitz, O. J. 2007. Predator diversity and trophic interactions. *Ecology*, **88**, 2415–2426.
- Seed, R. and Hughes, R. N. 1995. Criteria for prey size-selection in molluscivorous crabs with contrasting claw morphologies. *J. Exp. Mar. Biol. Ecol.*, **193**, 177–195.
- Sieben, K., Ljunggren, L., Bergström, U., and Eriksson, B. K. 2011. A meso-predator release of stickleback promotes recruitment of macroalgae in the Baltic Sea. *J. Exp. Mar. Biol. Ecol.*, **397**, 79–84.
- Smaldon, G. 1979. British coastal shrimps and prawns: keys and notes for the identification of the species. In *Synopses of the British Fauna (New Series)*, p. 126. Academic Press, London.
- Speckman, S. G., Piatt, J. F., Minte-Vera, C. V., and Parrish, J. K. 2005. Parallel structure among environmental gradients and three trophic levels in a subarctic estuary. *Prog. Oceanogr.*, **66**, 25–65.
- Taylor, A. C. and Spicer, J. I. 1987. Metabolic responses of the prawns *Palaemon elegans* and *P. serratus* (Crustacea: Decapoda) to acute hypoxia and anoxia. *Mar. Biol.*, **95**, 521–530.
- Weis, J. S. 2011. Invasion and predation in aquatic ecosystems. *Current Zoology*, **57**, 613–624.
- Wernberg, T., Thomsen, M. S., and Kotta, J. 2013. Complex plant–herbivore–predator interactions in a brackish water seaweed habitat. *J. Exp. Mar. Biol. Ecol.*, **449**, 51–56.
- Worm, B. and Myers, R. A. 2003. Meta-analysis of cod–shrimp interactions reveals top-down control in oceanic food webs. *Ecology*, **84**, 162–173.
- Yamada, S. B. and Boulding, E. G. 1998. Claw morphology, prey size selection and foraging efficiency in generalist and specialist shell-breaking crabs. *J. Exp. Mar. Biol. Ecol.*, **220**, 191–211.

Kohaliku ja võõrliigist kreveti ning võõrliigist kirpvähi toitumissuhted riimveelises ökosüsteemis

Ivan Kuprijanov, Jonne Kotta, Velda Lauringson ja Kristjan Herkül

Makrovetikad on väikestele selgrootutele, nagu kirpvähilised ja krevetilised, oluliseks elupaigaks. Kirpvähilised on rannikumeres tähtsad herbivoorid, samas kui krevetilised võivad käituda nii kiskjate kui ka herbivooridena. Hiljuti Läänemere põhjaossa jõudnud võõrliik elegantne krevett (*Palaemon elegans*) asustab kohaliku läänemere krevetiga (*P. adspersus*) sarnaseid elupaiku ja võib oletada, et elegantse kreveti invasioon muudab olemasolevaid toitumissuhteid. Elegantse kreveti mõjude väljaselgitamiseks uuriti eksperimentaalselt kohaliku liigi ja võõrliigi toitumissurvet makrovetikatele ning võõrliigist vöotkirpvähile (*Gammarus tigrinus*). Eksperimendi käigus ei avaldanud kumbki krevetiliik ega ka vöotkirpvähk makrovetikatele mõju. Krevettide esinemine suurendas drastiliselt kirpvähkide suremust, kuid kahe krevetiliigi toitumise intensiivsuses ei esinenud erinevust. Tulemustest võib järeldada, et võõrliigist krevett ei esinda Läänemere põhjaosas uut ökoloogilist funktsiooni, kuid üha laieneva leviku ja kasvava asustustiheduse tõttu võib elegantne krevett avaldada kirpvähkidele tugevamat kiskluse survet, kui seda suudab kohalik läänemere krevett üksi.

The Invasive Polychaetes (*Marenzelleria* spp.)

Impact of non-indigenous macrobenthic species on structural and functional diversity in the Vistula Lagoon (southern Baltic Sea)

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The American Comb Jelly (*Mnemiopsis leidyi*)

Ocean current connectivity propelling secondary spread of a marine invader across western Eurasia

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